PLANT PHYSIOLOGY

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ERRATA

Volume 24:

Page 1, last line, Ca should read Cu.

Page 728, first sentence should be the last paragraph in the section on Effect of Substrate Concentration.

Volume 25:

Page 282, error in alignment in table I. Should read:

1940	No potash added	Chlorotic tips, dried margins	0.094	94.0		
	Potash at 30 lbs. per acre	Leaf tip chlorosis	0.093	93.0		
	Potash at 240 lbs. per acre	Normal	0.100	100.0		
1945	No potash added	Normal	0.098	100.0	0.053	0.034
	No potash added	Frenched, few strapped leaves	0.188	191.8	0.112	0.051
1945	No fertilizer added	Normal	0.095	100.0	0.057	0.034
	No fertilizer added	Frenched, few strapped leaves	0.210	221.1	0.115	0.050

Page 307, line 35, KH_2PO_4 should read $H\,PO_3$.

Page 323, line 22, $Fe_2(So_4)_3$ should read $Fe_2\,(SO_4)_3$.

Page 410, line 38, 0.30 should read 0.80.

Page 421, Equation (3), $W = \int_{vb}^{va} PdV$ should read $W = \int_{va}^{vb} PdV$.

Page 430, line 26 should be transferred to follow line 36.

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In Memoriam

DENNIS ROBERT HOAGLAND

1884–1949

This volume of Plant Physiology
is dedicated to the memory of
Dennis Robert Hoagland

DENNIS ROBERT HOAGLAND

1884–1949

DENNIS ROBERT HOAGLAND

1884–1949

Dennis Robert Hoagland, Professor of Plant Nutrition in the University of California, a distinguished plant physiologist and leading authority in the broad field of plant and soil interrelations, died in Oakland, California, on September 5, 1949.

For over thirty years, Professor Hoagland's life and work has exerted a profound influence on the evolution of plant and soil science in America and abroad. But as with most great men, the totality of his achievement is greater than the sum of his impressive individual contributions. He personified the qualities of mind and character which led some to observe that the scientist with a broad outlook on his work and the world around him is among the finest products of western civilization.

D. R. Hoagland spent the first eight years of his life in the town of his birth, Golden, Colorado. In his own words "this was a small town, with all narrowness of small town life. Something of the frontier spirit remained, however. The history of Golden was linked especially with the development of mining in Colorado. The foothills and mountains were near and familiar. My home was one of moderate comfort according to the standards of the time and place." All of his later childhood was passed in the city of Denver, where, except for the first two years of grammar school, he received his primary and secondary education before entering college. He graduated from East Denver High School in 1903.

Even in his early years he possessed an extreme fondness for reading, a trait which was to remain with him for life. Again, to quote him—"At the age of nine or ten a most cherished Christmas gift was a dictionary. My reading was indiscriminate: novels, boy's stories, history. I was particularly attracted by stories which attempted to present scientific knowledge or speculation, such as 'Twenty thousand leagues under the sea'." In school he showed great promise by the uniformly excellent work in classes and a genuine interest in many studies extending beyond assigned work. Serious illness in childhood adversely affected his eyesight, and this handicapped his participation in sports.

It was during his high school days that he first acquired a definite interest in science "as a result of discussion of the theory of evolution which was condemned at that time by many ministers of the city; . . . my thinking was greatly stimulated by the ideas and points of view of biology with regard to man's place in nature."

Hoagland left Denver to enter the then new but already renowned Stanford University, where he elected chemistry as his field of study. He graduated from Stanford in 1907 and was elected that year to Phi Beta Kappa

and to the Chemistry Honor Society, now Phi Lambda Upsilon. An amusing anecdote of the Stanford period which he was fond of telling in later years was a "slightly exaggerated" report of his death in the great San Francisco earthquake in 1906, published with a suitable obituary by a Denver paper.

After graduation he first elected to go on with graduate work in chemistry at Stanford and was for one semester a graduate assistant in physical chemistry. In January of 1908, however, he took a fateful turn in his career by accepting a position as an instructor and assistant in the Laboratory of Animal Nutrition at the University of California. The new position brought him for the first time in professional contact with biology and took him to Berkeley, where, except for a few short gaps he was to spend the rest of his life. Perhaps it was highly significant for his future life-long activity in the field of plant nutrition that his introduction to biology was through the field of animal nutrition. He entered that field at a time when his training and skill as a chemist were welcome and useful but by no means decisive in gaining a thorough understanding of the essentials of nutrition. There were of course, during that period, large areas of empiricism and sheer ignorance regarding the effects of different foods on nutrition and health and this general atmosphere might have very well helped him in acquiring the outlook of a biologist which he so thoroughly displayed in his later work. He was spared the experience which some chemists undergo when first turning to biological pursuits—the failure to take into account the complexity of biological systems and the resultant predilection to oversimplification.

Hoagland remained in the field of animal nutrition and biochemistry from 1908 to 1913. In 1910 he joined the Consulting Referee Board of Scientific Experts of the United States Department of Agriculture. This board was established to study the effect on health of various chemicals used in food processing and as preservatives, a question of national concern raised by the administration of the then recent Pure Food and Drug Act. Hoagland acted as expert chemist and was associated with Dr. Alonzo Taylor in his work on the toxicity of aluminum, copper, and sulphur compounds. The experimental subjects were the so-called poison squad, a group of volunteer premedical students at the University of California and the University of Pennsylvania. His assignment took him to Philadelphia and made it possible for him to attend lectures as a graduate student in the University of Pennsylvania.

After the completion of the work of the Referee Board in 1912, Hoagland accepted a graduate scholarship to work in animal biochemistry with Professor E. V. McCollum in the Department of Agricultural Chemistry at the University of Wisconsin. This was another crucial event in his life. In later years Hoagland looked back on the year of graduate study under Professor McCollum as his real inspiration for a life devoted to scientific research. In 1913 he received his Master's Degree with a thesis on "The influence of sodium benzoate on the synthesis of urea." In the same year he

published with E. V. McCollum a series of three papers on the "Endogenous metabolism of the pig as modified by various factors." These publications profoundly altered the current ideas on an important phase of animal metabolism and are still quoted as an authoritative statement on the process involved.

In 1913 Hoagland had thus completed a period of five years of continuous work in animal nutrition and had become associated with an inspiring teacher and leader in the field. There would normally have been every expectation that he would continue in this field which was then on the eve of revolutionary advances. Circumstances intervened, however, and Hoagland abandoned the subject in which he had already amassed appreciable experience, to enter upon a new field of activity. For this, plant science will always remain indebted to Professor John S. Burd, then Head of the Division of Agricultural Chemistry in the College of Agriculture of the University of California. In 1913, Professor Burd invited Hoagland to come to Berkeley as an Assistant Professor of Agricultural Chemistry. Acceptance followed and with it the initiation of a scientific activity in a field in which he labored with devotion and distinction until the end of his days.

The appointment in the Division of Agricultural Chemistry of the University of California was a real opportunity to one who had already acquired an attachment for fundamental research. The department, under the leadership of Professor Burd, was chiefly interested in problems of fertilizers and soil chemistry. Practical and immediate as these questions appeared, Professor Burd had the vision to encourage the search for principles rather than *ad hoc* solutions which were thought by some to be attainable by a mere multiplicity of fertilizer field plots. A fortunate circumstance was also the early connection of Hoagland with teaching. It seems legitimate to speculate that the then large areas of ignorance in the field of plant and soil interrelations were sharply illuminated by penetrating questions from students.

The interruption of commerce in World War I focused attention on American dependence on German sources of potash fertilizers. There was considerable interest in exploring domestic sources of potassium and the giant kelps of the Pacific Coast were suggested as a possible source of supply. Hoagland undertook a systematic study of the inorganic and organic composition of kelps and reported his findings in three papers. His conclusions about the possibility of commercial exploitation of these organisms were not very encouraging but this research led him into a field of investigation in which he acquired a life-long interest and international renown: the accumulation of ions by plants. In 1915 he wrote in a paper dealing with the composition of kelps: "Of far greater interest to the plant chemist and physiologist is the study of the chemical composition and metabolism of these remarkable plants (rather than their economic importance). Their selective action on certain elements contained in sea water is very striking." He was particularly impressed with the remarkable ability of these plants to

accumulate potassium and iodine many times in excess of the concentrations found in sea water.

The second of his early research activities concerned itself with the effect of hydrogen ion concentration on plant growth. This was the era of "hydrogen ion concentration" in biology, when great expectations were centered around the introduction of the hydrogen electrode for the measurement of pH of biological systems. One of the accepted tenets of agricultural teaching in those days was that plants could only grow well at neutral or slightly alkaline reactions. It was with great surprise therefore that he discovered that barley plants made excellent growth in nutrient solutions at pH 5. It seemed that the chief importance of hydrogen ion was in relation to other ions rather than in its direct effect on the plant. It was also apparent that the inherent complexity of soils rendered untrustworthy many sweeping generalizations based on local conditions. To his orderly and critical mind it became clear that the complex problems of soil and plant interrelations must be studied by techniques which permitted rigid experimental control and the isolation of individual variables. This he expressed as follows: "Scientific research on soils and plants must place great reliance on the results of experiments conducted in the laboratory or greenhouse. The objection often raised to such investigations is that they are artificial and do not imitate field conditions. This is very properly the case, otherwise the requisite control would be absent. There can be nothing mysterious about field conditions. They are simply more variable and uncontrolled than soil cultures in pots or tanks."

He gave expression to his convictions about the value of artificial culture methods in plant research by developing to a very high degree the water-culture technique for growing plants, which has since, for over three decades served as a powerful and discriminating tool for studying fundamental problems in plant physiology at Berkeley. Under the influence of the soil solution studies conducted by Professor Burd, Hoagland devised a culture solution to imitate a soil solution obtained from a soil of proven fertility. This "Hoagland's solution" became, and remains to this day, a household term in laboratories of plant physiology throughout the world. The formulation of his culture solution was accompanied by a study of certain basic concepts such as concentration versus total supply of ions, specific proportions of nutrients and the like which underlie the use of nutrient solutions. He critically examined and rejected the idea of an "optimum nutrient solution," a point of view which was fully corroborated by later work.

It was inevitable that his early researches into plant and soil chemistry would lead him to a conviction that the study of soil and plant interrelations could be pursued most auspiciously by a closer union between workers in soil chemistry and plant physiology. In his own words: "Soil chemistry and plant physiology cannot be divorced and the practical art of soil management must have as its scientific basis a definite concept of the chemical and physiological interrelation between soil and plant." This point of view

already had strong advocates in the University of California College of Agriculture in the persons of the late C. B. Lipman, then Head of the Division of Soil Chemistry and Bacteriology, and J. S. Burd, Head of the Division of Agricultural Chemistry. In 1922 an administrative rearrangement in the College of Agriculture resulted in the merger of the two groups into a new Division of Plant Nutrition, and, with the blessing of the two Heads of the former Divisions, Hoagland was designated the Head of the new Division.

After the organization of the Division of Plant Nutrition, Hoagland returned with renewed vigor to the problem which attracted his attention in kelps—the absorption and accumulation of ions by plants. The quality of the immense contribution which he, with his collaborators and associates made to this fundamental process of plant physiology can only be judged against a background of the ideas and concepts which prevailed when his work got under way. There was no clear view as to how inorganic elements are absorbed by plants. Discussions of this subject were usually invoking such concepts as osmosis, permeability, antagonism, none of which as he later clearly demonstrated could explain the absorption, accumulation, and retention against a concentration gradient of anions and cations by plant cells. The prevailing confusion and uncertainty of thought at that period is illustrated by such contemporary theories as that the absorbing power of plants is contingent upon the reaction of cell sap and governed by the law of mass action. To explain potassium accumulation in plant cells it was sometimes assumed that this ion was precipitated in the vacuole.

Hoagland opened the study of ion absorption by using intact barley plants. Promising as the early results were, he readily perceived the advantages of working with a unicellular organism from which relatively pure vacuolar sap could be obtained. At the suggestion of the late Dr. Nathaniel Gardner, he selected the fresh water alga, Nitella. This work clearly demonstrated that absorption of ions by plants is a metabolic process and not a question of simple permeability. For example, absorption and accumulation of bromide could take place in Nitella only under the right metabolic conditions—proper temperature and illumination. The accumulated ions were not precipitated in the sap but remained as free ions, the concentration of electrolytes in the Nitella sap being twenty-five times greater than that in the surrounding medium. Although the concept of "ion antagonism" had no strict applicability to the normal process of nutrient absorption, there was clear evidence of reciprocal effects of one ion on the absorption of another, as, for example, potassium on calcium, and nitrate on phosphate. Here was painted in bold strokes an outline of a theory which at once correlated a host of observations in the field and laboratory, and made it possible to approach an infinitely complex subject with principles instead of mere empirical observations.

The next important phase of his researches on ion accumulation brought him closer to the system which was uppermost in his mind—the absorption

of nutrients by the roots of higher plants. The broad outlines of a theory were already available from his previous work. What was needed was the formulation of an adequate experimental approach to the different system. In the solution of this problem, Hoagland displayed in a most striking manner his remarkable gift for resolving a complex question into its essential components and arranging them unerringly in an order of relative importance. He was always mindful of the inherent complexity of biological systems, their adaptive properties to varying conditions, and hence was distrustful of the spectacular short-cut or facile generalization unless it was supported by solid experimental evidence. As far as salt absorption by roots was concerned, he decided in the late 1920's that the most important prerequisite for future work was the development of a technique which would make available biological material of a definable and reproducible physiological status. Only in this manner could the various conflicting observations be resolved and a systematic effort directed toward the elucidation of factors underlying salt absorption by roots.

He concluded that the fundamental questions of ion absorption by root cells can be studied most effectively by eliminating, during a brief experimental period, the complications of shoot and root relationships. He concentrated therefore on excised roots, obtained from plants grown under experimental conditions designed to yield, subsequent to the removal of shoots, root systems with a high capacity for salt absorption. Among the essential conditions for a high capacity for ion absorption by roots were a low salt and a high carbohydrate status. Under these conditions the excised roots were found to be "the most active salt-accumulating system so far (1936) investigated."

Once the tedious but indispensable job of evolving a suitable physiological technique was completed, progress was rapid and gratifying. An impressive array of data was amassed over the years on the influence of temperature, light, hydrogen ion concentration, ion selectivity, concentration of anions and cations, differences in rates of absorption, and the effect of one ion on the absorption of another ion of the same or opposite charge. A striking correlation was obtained between the supply of oxygen and the absorption of salts by roots against a concentration gradient. A solid scientific foundation was thus laid for the understanding of the importance of aeration in soils and for the interpretation and prediction of a multiplicity of plant responses to fertilization treatments, and other chemical changes in the soil.

The final phase of his investigations on absorption of ions occurred just before and during World War II. With excised barley roots he studied the effect of reversing the external concentration gradient on ion entry and the influence of inhibitors on ion accumulation. He also returned to Nitella, this time employing radioactive isotopes—a sensitive technique which permitted the separation of the two stages of ion absorption by cells: from the external medium into the cytoplasm, and from the cytoplasm into the

vacuole. The fundamental conclusion, which he and his colleagues reached almost two decades earlier, that ion accumulation is a metabolically linked process, was confirmed. With the use of radioisotopes it was possible to show that ions first accumulate in the cytoplasm but subsequently they migrate to the vacuole by a process akin to secretion, until the vacuolar concentration becomes higher than that in the cytoplasm and decidedly higher than that of the external dilute solution.

Dominant as his life-long interest in the process of ion absorption was, it by no means prevented him from pursuing with vigor other phases of plant nutrition to which he, with his associates, made outstanding contributions. He studied by means of different techniques the upward movement and distribution of inorganic solutes in the plant. In the late 1930's, when radioactive isotopes from the cyclotron became available, he recognized at once their serviceability and used them in resolving some of the hitherto perplexing uncertainties as to the path of ion movement in higher plants. In a series of experiments on the relation between the movement of solutes and absorption of water by plants, he again demonstrated with masterful clarity the extent to which the two are related as well as independent of each other.

Hoagland was keenly aware of the immense importance for physiological studies of the control of light, temperature and humidity as well as of the culture medium. He was instrumental in securing an installation for the control of environmental factors in Berkeley, it being one of the first in America. He envisaged "the development of a quantitative plant physiology in relation to mineral nutrition" when it will be possible "to do exactly the same thing twice."

A tribute to the catholicity of his interests and the breadth of his understanding is that preoccupation with precise laboratory experiments and insistence, when needed, on an exact though "artificial" culture condition, in no way diminished his appreciation of the importance of direct studies on soils and on crops grown in soil. In his own words: "Viewing the field as a whole, the attack on problems of plant nutrition assumes wide dissimilarity of method. At one time, the appropriate tool may be a spade or a soil auger; at another time a highly specialized and refined tool of physics or chemistry may be required. For some purposes, crude measurements in terms of pounds or ounces may suffice; for other purposes, a thousandth of a milligram or even much less than that is of consequence." This heterogeneity of experimental method, was necessary, he felt, because of the extraordinary complexity of the plant-soil-atmosphere system which the investigator of plant nutrition explores. To quote him again: "Inherent in the green plant itself are all the complexities common to living organisms and to these must be added the complexities of the soil medium in which the plant is anchored and finds some of the substances essential for its nourishment. . . . Finally, the whole system of plant and soil is subject to the influence of another constellation of factors in the atmospheric environment—

light, temperature, humidity, rainfall, air movement, carbon dioxide and other gaseous components of the atmosphere."

His own work on problems of soil chemistry insofar as it affects the nutrition of crops grown in the field, was especially concerned with zinc, potassium, and phosphate deficiencies of fruit trees in California. In this he entered, in the late 1920's, into a harmonious cooperation with Professor W. H. Chandler of the Pomology Division, and the resultant diversified investigations which embraced field, greenhouse, and laboratory experimentation will stand out as models of cooperation among different divisions in an agricultural experiment station. In a concerted attack on a complex problem, each division contributed what it was best equipped and qualified to do. The result of this collaboration was the clear identification of "little leaf" of fruit trees as a zinc deficiency and its reproduction under controlled greenhouse conditions. The importance of these studies to California agriculture is shown in the fact that at the present time, zinc is second only to nitrogen as a fertilizer element for orchard crops. The investigation of potassium nutrition of fruit trees had a bearing on "prune dieback," another nutritional disease of fruit trees in California. An intensive chemical investigation followed into the question of potassium absorption in relation to replaceable, non-replaceable, and soil solution potassium. His interest in the nutrition of fruit trees continued, and in 1940 he produced, under controlled conditions, the first molybdenum deficiency symptoms in fruit trees. It was also under his influence and encouragement that intensive studies into other aspects of micronutrients have been undertaken in the Division of Plant Nutrition.

In the middle 1930's, immense public interest was aroused in the commercial possibilities of nutrient solution techniques for crop production. To one who, like Hoagland, had been using nutrient culture techniques for over a quarter of a century for experimental purposes, this newly aroused enthusiasm appeared to be wholly without scientific foundation. He preferred to remain aloof from this development and to await its expected demise, but to save embarrassment to his institution, he undertook, in response to a direct request, a careful study of the inherent productive capacity of a fertile soil as compared with artificial nutrient media. The results, which were the only ones of their kind available, not only provided a scientific basis for evaluating diverse factors, but also served as a foundation for the preparation of a popular circular which was distributed in response to tens of thousands of requests which came to the University of California. It is safe to say that this publication exerted great influence in wide circles in restoring a proper perspective with regard to crop production in soil.

In summarizing Hoagland's contribution to plant nutrition, it is pertinent to record that he did not regard the field as a unified discipline. In his own words: "An essential condition in the field of study of Plant Nutrition . . . is the uniting together, although not according to any rigid pattern, of persons of varied technical interests, in a research program that has a com-

mon objective." While he himself was mainly engaged in what is known as mineral nutrition of plants, he was keenly aware of the importance of the biochemical aspects of plant nutrition. He wrote "The inorganic elements derived from the soil constitute only a small percentage of the dry weight of a plant and one of the most important objectives of research in plant nutrition should be an understanding of the mechanisms by which these inorganic components become directly incorporated into the organic compounds synthesized by the plant, or activate the enzymes which catalyze the syntheses and breakdown of organic compounds." Under his leadership the Division of Plant Nutrition became an outstanding center of research, encompassing a diversity of investigations seldom encountered within the confines of a rather small administrative unit. He was always mindful, however, of "the broad common objective" and exerted great personal influence in guiding the thoughts and interests of his associates into channels which would ultimately lead to the common goal.

Hoagland's entire life as a productive scientist was spent in association with agricultural research. He had a profound faith in the importance to agriculture of fundamental research, without thought and expectation of immediate practical returns. He was fully aware of the often wide gap between the laboratory result obtained under controlled conditions and the field application complicated as it is by a multiplicity of factors. He sought principles in the laboratory to help in solving the practical problems of the farmer in the field, but was never prone to seek in laboratory observations a facile generalization which would solve field problems with dazzling simplicity. He was conscious not only of the promise but also of the limitations of science in agriculture, and his thoughts on this subject ranged beyond mere material achievements. His philosophy is best described by two quotations which he himself selected in a public address as best representing his own views on these matters. The first is from Sir Daniel Hall: "Great are the achievements and still greater the possibilities of agricultural research, but we must recognize that there are limitations to the effect of science upon agriculture which do not hold for the other industries. In the first place, in agriculture we are dealing with a living organism and the amount of control that we have obtained over plant or animal, over that stubborn essence we call life, is far less than we can exercise over inanimate nature, over iron or cement, over even the ether or the atom. When we attack vital problems we find that we cannot speed up processes or enlarge the unit in the way we can deal with the dynamo or spinning frame. It still takes the wheat plant six or nine months to develop, and cows bring forth their calves neither more quickly nor more numerously for us than they did for Abraham. We see no way of growing three or four crops a year under temperate climatic conditions. The organisms we are dealing with will go through their cycle and you cannot hurry them. When you start hustling you find you let in secondary troubles of all sorts."

The second quotation is from Sir John Russell, the now retired Director

of the Rothamsted Experiment Station: "And that leads to my last point. What is the purpose of it all? Team work, cooperation, the great expenditure of time and money now being incurred in agricultural science and experiment—these are justified only if the end is worthy of the effort. The nineteenth century took the view that agricultural science was justified only insofar as it was useful. This view we now believe to be too narrow. The practical purpose is of course essential; the station must help the farmer in his daily difficulties—which again necessitates cooperation, this time between the practical grower and the scientific worker. But history has shown that institutions and investigators that tie themselves down to purely practical problems do not get very far; all experience proves that the safest way of making advances, even for purely practical purposes, is to leave the investigator unfettered. Our declared aim at Rothamsted is to discover the principles underlying the great facts of agriculture and to put the knowledge thus gained into a form in which it can be used by teachers, experts, and farmers for the upraising of country life and the improvements of the standard of living.

"This wider purpose gives the investigator full latitude, and it justifies an investigation whether the results will be immediately useful or not—so long as they are trustworthy. For the upraising of country life necessitates a higher standard of education for the countryman; and education based on the wonderful book of Nature which lies open for all to read if they but could. How many farmers know anything about the remarkable structure of the soil they till, of its fascinating history, of the teeming population of living organisms that dwell in its dark recesses; of the wonderful wheel of life in which the plant takes up simple substances and in some mysterious way fashions them into foods for men and animals and packs them with energy drawn out of the sunlight—energy which enables us to move and work, to drive engines, motor-cars, and all the other complex agencies of modern civilization? No one knows much of these things; but if we knew more, and could tell it as it deserves to be told, we should have a story that would make the wildest romance of human imagination seem dull by comparison and would dispel forever the illusion that the country is a dull place to live in. Agricultural science must be judged not only by its material achievements, but also by its success in revealing to the countryman something of the wonder and the mystery of the great open spaces in which he dwells."

In his personal relationships Hoagland's chief characteristic was his integrity and objectivity of outlook. He was the not altogether common scientist who carried the scientific mode of thinking outside his own specialty. In the seventeen years during which the writer was associated with him as a student, colleague, and friend, he never heard him once discuss a person or an issue, scientific or otherwise, except in a fair and objective manner. He approached his students with confidence and good will, always crediting them at the start with ability and industry.

He was an ideal collaborator in scientific research, never seeking dominance for his own point of view but always inviting a free flow of ideas. He was meticulously careful in giving credit where credit was due and was generous in acknowledging the contributions of his associates. He was a master of clear exposition and had a real feeling for style. Although writing came to him easily, he always preferred to defer publication of results until the evidence was carefully assembled and checked.

As an administrator of a department he was anxious not to fritter away his time and energy on the minutiae of scientific housekeeping and administrative procedure, but sought to discharge his responsibility to the University by concentrating on the encouragement and development of the broad aspects of his field of science.

Hoagland's objectivity of outlook and keenness of judgment were always at their best in evaluating scientific evidence. In these matters he had the "four things" which Socrates said, "belong to a judge: to hear courteously, to answer wisely, to consider soberly, and to decide impartially." He applied the same critical standards to his own work as to that of others. He accepted no blanket authority in science, and expected every single piece of research to stand on its own merits regardless of its source. The writer recalls a characteristic incident in a graduate seminar: A student attempted to add emphasis to a certain point by remarking that a particular conclusion had the approval of a certain "big shot" in this field. Hoagland at once challenged the remark by saying that "there are no 'big shots' in science, there are only people who have a more consistent record than others of bringing forth good data."

It was only natural that his accomplishments, his qualities of mind and character, gained him wide recognition and many honors. His counsel was sought and valued within his own University, to which he gave unstintingly of time and effort in many arduous administrative assignments, usually at the expense of his own leisure and in later years at the expense of his health. In 1934 he was elected a Member of the National Academy of Sciences. The American Society of Plant Physiologists, which was his closest scientific affiliation, bestowed upon him its highest honor by granting him, in 1929, the first Stephen Hales Award in recognition of his outstanding research contributions. He was also elected President of the Society and occupied other important offices in it.

The high esteem in which he was held by plant physiologists throughout the world is attested by his recent appointment as President of the Section of Plant Physiology at the forthcoming VIIth International Botanical Congress to be held in Sweden in 1950. Unfortunately, poor health forced him to decline this international honor.

Many other societies in which he held membership honored him by election to presidency: Western Society of Soil Science (1924); Western Society of Naturalists (1931); Botanical Society of America, President of Pacific Division (1929); American Association for the Advancement of

Science, President of the Pacific Division (1941). He served as Consulting Editor for Soil Science, American Journal of Botany and Plant Physiology. He took a prominent part in the organization of the Annual Review of Biochemistry, serving from its very inception on the Advisory Board and later on, on the Board of Collaborators for the U. S. Department of Agriculture Salinity Laboratory at Riverside, California, and the Soil, Plant and Animal Nutrition Laboratory at Cornell University.

In 1940, the American Association for the Advancement of Science awarded him the $1000 prize for an outstanding paper presented at the Philadelphia meetings. In 1942 he was invited by Harvard University to give the John M. Prather lectures. The lectures were later published in book form, and constitute the only book which he found leisure to prepare. His own colleagues in the various Faculties, bestowed upon him the highest honor within the University, by appointing him in 1942, Faculty Research Lecturer.

Hoagland was never in robust health, but a strong sense of duty compelled him to carry on without a thought for his own personal well-being. The last four years of his life were marred by serious illness. He carried on with courage and determination up to within the last few months of his life when his eyesight failed him almost completely. This latter blow was the severest of all, for throughout his life he enjoyed reading on a wide variety of subjects. He had a keen interest in contemporary ideas, whether in science, politics, or literature. It was characteristic of the man that once he gave up hope of regaining his health, he requested retirement from the University, ignoring the serious financial adjustment that such a voluntary step would involve. He was officially retired on July 1, 1949, and died two months later.

In 1920, he married Jessie A. Smiley. She died suddenly of pneumonia in 1933. He was left with the responsibility of bringing up three young boys. His three sons, his mother and a brother survive him.

The scientific influence of Professor Hoagland's life has not come to an end with his death, but will continue through the deep impression which he made on the minds and hearts of his students and friends.

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THE INFLUENCE OF CALCIUM ION ACTIVITY IN WATER CULTURES ON THE INTAKE OF CATIONS BY BEAN PLANTS[1]

C. H. WADLEIGH AND C. A. BOWER[2]

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It is becoming increasingly evident that the adverse effect of "alkali soils" (**14**) on plant growth is due in part to the decreasing availability of calcium associated with increasing proportions of sodium upon the exchange complex of the soil. RATNER (**12**) presents data to show that a given level of adsorbed Ca is less exchangeable in the presence of adsorbed Na than when H, Mg, and K are the complementary cations adsorbed. His evidence indicates (**11, 13**) that the energy of retention of Ca by soil colloids is sufficiently high in the presence of a relatively high level of adsorbed Na to prevent not only the intake of Ca by the plant but to actually remove Ca from the plant with the resultant death of the plant tissues from Ca starvation. He observed (**12**) that wheat seedlings failed to take in calcium from soil with an exchangeable-sodium percentage of 40. VAN ITALLIE (**16**) found that growth of Italian rye grass was impaired by an exchangeable-sodium-percentage in the soil of 26 and prevented by one of 50. THORNE (**15**) observed that the ability of tomatoes to take in calcium was reduced when the exchangeable-sodium-percentage was 40 or higher.

BOWER and WADLEIGH (**3**) employed cultures with nutrients adsorbed on ion-exchange resins (**1**) to ascertain the nutritional effects of varying exchangeable-sodium-percentages on growth and cation accumulation by four species of plants. They found that different species varied widely in their response to increasing proportions of adsorbed sodium on the cation exchange complex. Dwarf red kidney beans were especially sensitive to this condition. Increasing exchangeable-sodium-percentages, with concomitantly decreasing exchangeable calcium and magnesium percentages, were effective in inducing marked decreases in the accumulation of Ca and Mg in tops and increases in sodium accumulation. However, the most striking effect of the substrates upon cation accumulation was found in the roots. The content of Ca and Mg per unit weight of root tissue was constant for

[1] Contribution from the U. S. Regional Salinity and Rubidoux Laboratories, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Dept. of Agriculture, Riverside, Calif., in cooperation with the eleven western states and the Territory of Hawaii.

[2] Principal Chemist and Soil Scientist, respectively.

all treatments. On the other hand, even though exchangeable-potassium-percentage was constant for all treatments, there was a marked decrease in potassium accumulation in the roots with increasing exchangeable-sodium-percentage. The trend of potassium content of the roots was more closely related to growth than that of any other component studied. This relationship was regarded as a response to the phenomenon pointed out by JENNY and AYERS (**10**) to the effect that potassium adsorbed on soil colloids becomes less exchangeable as the adsorbed complementary ions are shifted from predominantly Ca to Na. Furthermore, potassium moves readily into the tops of bean plants whereas sodium tends to be restricted to the roots (**7**). BOWER and WADLEIGH (**3**) found very high accumulations of Na in the roots of plants at the higher exchangeable-sodium-percentages indicating a certain degree of cationic replacement in the roots. Thus, there was evidence that the very poor condition of the plants at the higher exchangeable-sodium-percentages was related to serious potassium deficiency in the roots, as well as Ca deficiency in the tops.

As a result of the aforementioned study, the question immediately arose as to what extent the plant responses observed in the presence of an ion exchange medium could be duplicated by plants grown in solution cultures varying as to calcium availability in the absence of ion adsorbents. This paper presents the results of a study of the problem.

Experimental procedure

Previously unreported data from the work of BOWER and WADLEIGH (**3**) are set forth in table I and provide the basis for the nutrient solutions used in the present study.

TABLE I

AVERAGE CATIONIC COMPOSITION OF EFFLUENT OF CULTURES USED BY BOWER AND WADLEIGH (3)

EXCHANGEABLE Na IN SUBSTRATE	CATIONS IN EFFLUENT			
	Ca	Mg	K	Na
%	*m.e./l.*	*m.e./l.*	*m.e./l.*	*m.e./l.*
0	5.4	2.6	3.1	2.5
15	3.9	1.7	2.8	11.6
30	3.1	1.3	2.5	15.9
45	3.0	1.0	2.5	24.3
60	2.9	0.9	2.6	29.4
75	2.6	0.8	2.5	28.8

It is evident that the concentrations of the cations K, Ca, and Mg in the effluent were ample for normal plant growth if a solution of comparable composition had been used in a solution culture study. Furthermore, the highest amount of sodium present should not have been specifically toxic to bean plants (**7**). Thus, in order that the above mentioned plant responses could have taken place, the cationic exchange medium must have affected the

distribution and activity of the cations in the diffuse double layer about this adsorbent, consequently affecting the relative activities of these cations and their qualitative distribution on the absorbing surface of the roots. Hence, nutrient solutions were prepared for the present study having a wide variation in calcium activity in the absence of added sodium chloride and in the presence of an addition of 24 m.e./l. NaCl. All nutrient solutions contained the following components:

5 m.e./l.	KNO_3
0.5 "	KH_2PO_4
2 "	$MgSO_4$
1.0 p.p.m.	Fe as FeCitrate
0.5 "	Mn as $MnSO_4$
0.5 "	B as HBO_3

The variation in calcium additions is shown in table II. The calculation of the activity coefficients for Ca was by means of the formulation of Debye and Hückel as given by GLASSTONE (9, p. 940–943).

Four 8-day-old dwarf red kidney bean seedlings were transferred to each 5-gallon crock of adjusted culture solution on April 30, 1948. Each of the

TABLE II

CONCENTRATION OF $CaCl_2$ AND NaCl IN NUTRIENT SOLUTIONS TOGETHER WITH THEIR IONIC STRENGTH AND THE ACTIVITY OF THE CALCIUM ION

SOLUTION DESIGNATION	$CaCl_2$ ADDED *m.e./l.*	NaCl ADDED *m.e./l.*	IONIC STRENGTH	ACTIVITY COEFFICIENT FOR Ca, a_{Ca} @ 25° C.	ACTIVITY OF Ca $a_{Ca} \times 10^3$ @ 25° C.
4	4		.0155	.559	1.22
1	1		.0110	.611	.31
1/4	0.25		.0099	.628	.079
1/8	0.125		.0097	.631	.039
S4	4	24	.0395	.394	.79
S2	2	24	.0365	.408	.41
S1	1	24	.0350	.416	.21
S1/2	0.5	24	.0342	.420	.11
S1/4	0.25	24	.0339	.422	.053
S1/8	0.125	24	.0339	.424	.027

10 nutritional treatments was replicated three times. Each culture was vigorously aerated by means of a carbon-tube aerator. The cultures were equipped with constant level devices so that when fresh solution was added each morning, any excess would flow off from the bottom of the culture jar. Four quarts of fresh nutrient solution were added to each culture each morning except during the last week of the experiment when six quarts were added daily. This was done to prevent extreme depletion of the prescribed levels of calcium. The extent of depletion which did take place is illustrated by the data in table III. Consequently, the Ca concentrations shown in table III should be taken as more nearly indicative of the conditions affecting

plant growth than those given in table II. The rapid rate of replenishment helped to maintain the pH of the solutions at 5.6–6.0.

The plants were harvested on May 19, 1948, weighed, and separated into leaves, stems, and roots. The roots were thoroughly washed in distilled water. The dried tissue was ground in a Wiley mill and wet-ashed by the method of GIESEKING *et al.* (**8**). The cationic composition of the dry tissue was determined by methods given in reference (**14**) with a modification (**5**) of the uranyl zinc acetate method for Na in plant material.

TABLE III

CONTENT OF Ca IN CULTURE SOLUTIONS JUST BEFORE BEING REPLENISHED WITH FRESH SOLUTION ON MAY 12, 1948

Ca IN ORIGINAL SOLUTION	Ca IN SOLUTIONS JUST BEFORE REPLENISHMENT	
	WITHOUT NaCl	NaCl ADDED
m.e./l.	*m.e./l.*	*m.e./l.*
4	4.08	4.14
2		2.23
1	.98	1.18
0.5		.59
0.25	.19	.20
0.125	.06	.06

Observations

The growth responses of the plants are shown in figure 1. It appears that maintenance of the Ca level at 0.5 m.e./l. was adequate for these bean plants both in the presence and absence of added NaCl. Addition of NaCl to the cultures did not vary relative growth response at the different levels of Ca nutrition. Tops of plants with added NaCl were only 58% as heavy as those without this salt. This decrease in growth was undoubtedly the result of decreased water availability from the increase in osmotic pressure of the nutrient solution since addition of 24 m.e./l. of NaCl increased osmotic pressure of these solutions by one atmosphere. The observed growth depression accompanying the addition of 24 m.e./l. NaCl to the substrate is much greater than that reported by GAUCH and WADLEIGH (**7**) for dwarf red kidney beans grown in comparable nutrient solutions. This latter study was carried on in December under relatively low light intensity. GAUCH[3] found that the relative growth depression with beans on saline substrates was much greater during the spring months with higher light intensities than during the winter when visible radiation was low.

The only plants which showed symptoms of malnutrition at the conclusion of the growth period were those subjected to the lowest level of calcium supply. The symptoms consisted largely of a slight chlorosis near the margins of the older leaves. These plants had also showed some of the symptoms of moisture stress during the heat of the day.

[3] Gauch, H. G. Unpublished data, U. S. Regional Salinity Laboratory.

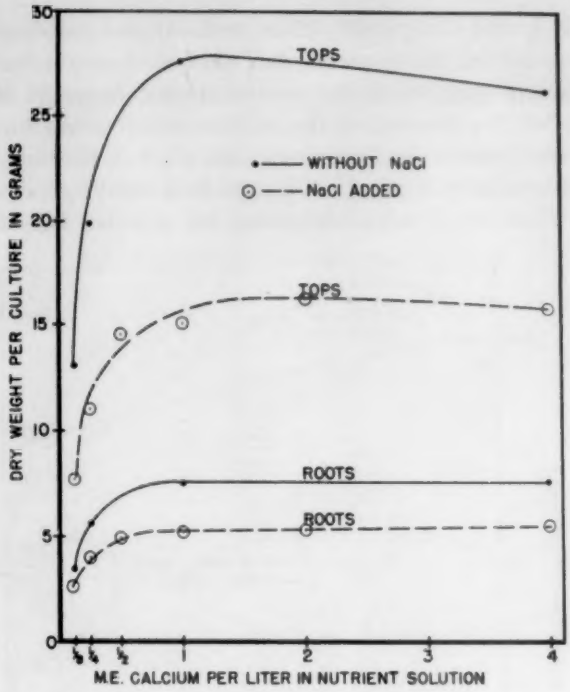

FIG. 1. Growth response of plants.

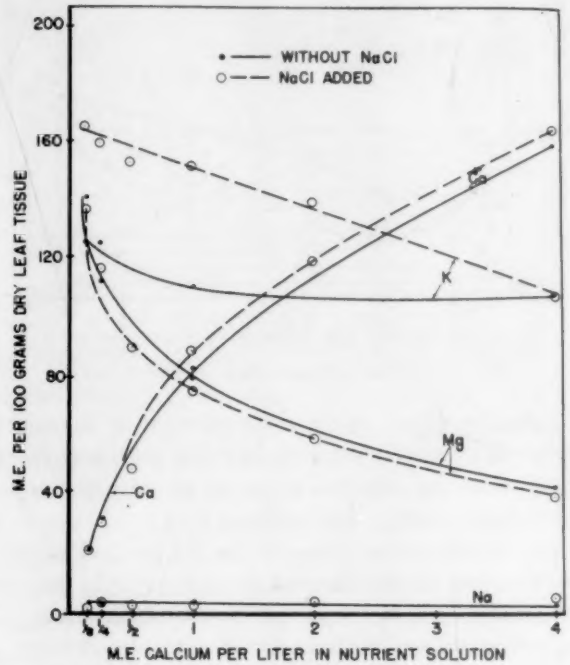

FIG. 2. Cation accumulation in leaf tissue.

Figures 2, 3, and 4 show the cation accumulation in terms of m.e./100 gms. dry tissue for the leaves, stems, and roots of these plants.

The concentration of Ca in the various tissues increased directly as the concentration of this element in the culture solution was increased. The most pronounced increase in Ca content took place in the leaves; those from plants on cultures with 4 m.e./l. Ca contained eight times as much Ca in the tissue as those on cultures containing 1/8 m.e./l. The addition of 24

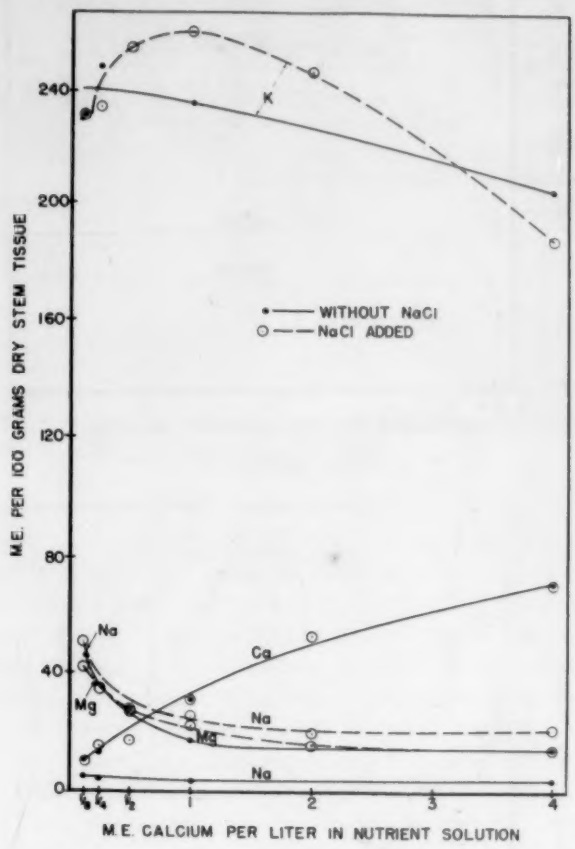

FIG. 3. Cation accumulation in stem tissue.

m.e./l. of NaCl had no effect on Ca concentration in the plant tissue at a given level of calcium supply, even though the resultant increase in ionic strength of these solutions caused a lowering of calcium ion activity at respective levels of Ca addition. (See table II.)

There was practically no variation in the Mg content of root tissue with variations in Ca supply in the absence of added NaCl; but when this salt was added, the general level of Mg in root tissue was lowered. The addition of NaCl to the substrate was not associated with any definite effect on the Mg content of tissue of the shoot. There was in the leaves and stems, how-

ever, an unusually high accumulation of Mg at the lower levels of Ca supply. This accumulation may have been sufficient to result in Mg toxicity in line with GAUCH's (6) observation that magnesium toxicity symptoms readily develop in bean plants on calcium deficient cultures.

Addition of NaCl to the culture solution was accompanied by a high accumulation of Na in the root tissue. There was a definite trend for the Na content of root tissue to decrease with increasing concentration of Ca

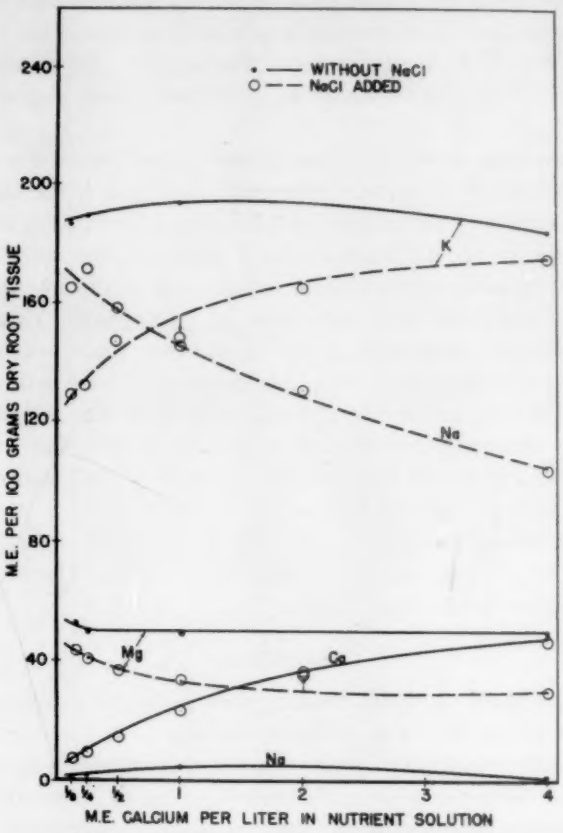

FIG. 4. Cation accumulation in root tissue.

in the culture solution. In the stems, there was a moderate tendency for Na to accumulate on addition of NaCl to the substrate. In confirmation of the observations of GAUCH and WADLEIGH (7), the addition of 24 m.e./l. to the water cultures had no effect on the Na content of leaf tissue of beans. This species exhibits a remarkable mechanism for accumulating Na in the roots while preventing its accumulation in the leaves.

The potassium content of all tissues in all treatments was unusually high. The concentration of this element in the root tissue showed little variation with varying levels of Ca supply in the absence of added NaCl. Addition of NaCl to the culture solution was accompanied by a decrease in the K

content of root tissue; and there was a marked tendency in the latter series of plants for K content of roots to increase with increasing levels of Ca supply. In other words, there was evidence of a reciprocal relation between the contents of Na and K in the root tissue. This does not imply constancy of cation equivalence in unit weight of tissue, however, since the dry tissue of roots from plants receiving added NaCl contained an average of 90 m.e./100 gms. more cations than did that from plants without added NaCl.

The stem tissue of the plants grown in water cultures was phenomenally high in potassium, and there were only minor variations in the concentration of this element with wide variations in Ca supply. There was some tendency for the K content to be higher in stem tissue from plants subjected to added NaCl.

The average concentration of potassium in leaf tissue was considerably lower than that in stem tissue. Whereas, there was little variation in K content in leaf tissue from plants without added NaCl as a result of variation in Ca supply, the addition of NaCl to the culture solution was associated with appreciable higher concentrations of K in the leaf tissue together with a marked negative trend with level of Ca supply. Thus, it appears that the observed accumulation of Na in the root tissue was conducive to the partial displacement of K from this tissue with concomitant accumulation in the leaf tissue which was virtually devoid of Na. Correspondingly, the trend of potassium accumulation in leaf tissue with varying Ca supply in the NaCl series of plants was just the converse of the trend in potassium accumulation in root tissue in this series.

These data are supporting evidence for a reciprocal relationship in the accumulation of the four cations studied among three tissues of the bean plant on variations in calcium supply.

Discussion

In this experiment, a comparison is made between cation accumulation in bean plants grown in water cultures varying as to calcium activity and that of bean plants grown on cultures containing nutrients adsorbed on ion exchange resins with Ca activity theoretically varied by means of varying exchangeable-sodium-percentages. This is shown in figure 5. The solid lines show the levels of accumulation within the dry tissue of the tops (leaves and stems) of the four cations with varying levels of Ca supply. The comparable values for the plants grown on cultures with ion exchange resins are shown by the dotted lines. The arrows above the abscissa show the levels of Ca found in the effluent from cultures adjusted to various exchangeable-sodium-percentages indicated by the numbers adjacent to the arrows. The values for Ca are taken from table I.

It is evident that use of the Ca concentration of the effluent from the ion-exchange resin cultures as a basis for plotting the trends in accumulation of the four cations presents a misleading picture in the light of the results from the water culture study. That is, Ca concentration of the effluent was not a

valid measure of calcium availability from the ion exchange resins if Ca accumulation from water cultures is taken as a standard.

The data show that addition of NaCl to the culture solution had no significant effect on Ca accumulation in bean tissue when various levels of Ca supply were maintained in water cultures even though the increased ionic strength of the solutions effects a decrease in calcium ion activity. Furthermore, the data on Ca accumulation by bean plants given by GAUCH and WADLEIGH (7) fit in very well with that here presented even though the

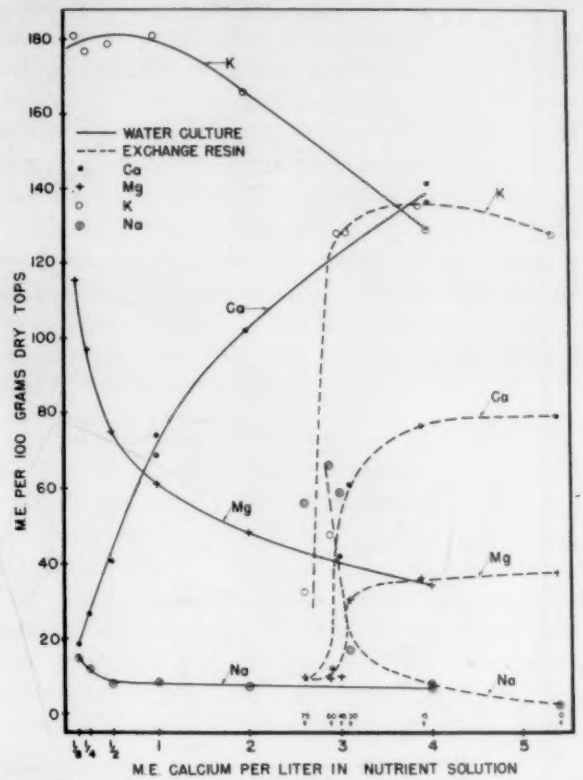

FIG. 5. Comparison of cation accumulations in tops of two groups of plants. Values for plants grown on exchange resins based on calcium concentration of effluent from these cultures.

respective groups of plants were grown during different seasons. It is reasonable, therefore, to take the curve showing Ca accumulation in the tops as an index of Ca availability in the substrate. This was done in figure 6. The arrows on the Ca curve designate the various levels of Ca accumulation in the tops of bean plants grown on cultures with ion exchange resins. The figures adjacent to the arrows show the exchangeable-sodium-percentage in the various cultures. By projecting these intercepts on the Ca-curve vertically to the abscissa, a set of values are derived which show the theoretical level of Ca that would need to be maintained in water cultures to effect the

levels of Ca accumulation shown by the tissue from plants grown with ion-exchange resins. Correspondingly, the levels of accumulation of the other three elements in this latter group of plants are also shown as functions of this theoretical level of Ca supply. The derived trend in Mg accumulation is reasonable on the grounds that percentage of exchangeable Mg decreased with increase in exchangeable-sodium-percentages, thereby effecting an appreciable range in Mg availability. The values for Na accumulation may also be readily explained on the basis of percentage of exchangeable Na.

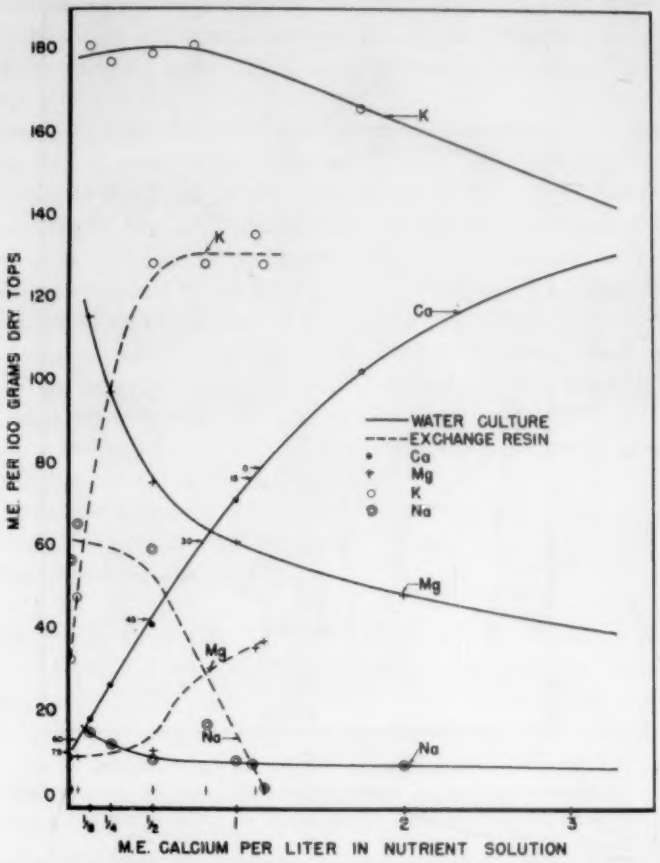

FIG. 6. Comparison of cation accumulations in tops of two groups of plants. Values for plants grown on exchange resins based on extrapolated calcium supply indicated by calcium content in tops.

On the other hand, even though potassium was supplied at constant levels for all treatments in the two respective types of culture, quite divergent trends were observed for potassium accumulation. Whereas, accumulation of potassium tended to approach a maximum at the lower levels of Ca supply in water culture, the opposite effect was very pronounced with the plants grown on ion exchange resins. This seemingly anomalous response as re-

gards accumulation of potassium was not predictable from the results of the water culture study involving varying levels of Ca or from the composition of the effluent from the ion-exchange resin cultures.

ARNON and MEAGHER (**2**) have discussed the availability to plants of ions adsorbed on ion-exchange resins and have emphasized especially the relative unavailability of adsorbed Ca and Mg. BOWER and WADLEIGH (**3**) also noted this effect, and that it was enhanced by increasing proportions of adsorbed sodium. That is, the complementary ion effect is operative on exchange resins. A given proportion of potassium adsorbed on a soil colloid is more easily released if the complementary ion is calcium rather than sodium (**4, 10**). The ease of release of the various cations is $Na > K > Mg > Ca$. Consequently, increasing exchangeable-sodium-percentage theoretically decreases ease of release of K as well as Ca and Mg. Decreasing potassium availability did not take place in the water culture study. Therefore, a water culture study based on varying availability of calcium with the assumption that Ca availability was the principal factor involved in the study with ion-exchange resins was not successful in simulating the responses in the latter study. This consequence is interpreted as being due to the operation of the complementary ion effect, particularly in its action on the availability of potassium in the presence of relatively high proportions of adsorbed sodium.

Summary

Red kidney bean plants were grown in aerated water cultures varying as to calcium supply in the presence and absence of added NaCl. Accumulation of cations in the various parts of the plant was ascertained. This was compared with similar data from bean plants grown on substrates with nutrients supplied on ion exchange resins, with exchangeable sodium percentage being the primary variant but with exchangeable Ca and Mg percentage varying reciprocally. The operation of the complementary ion effect on the adsorptive surface induced a marked deviation from the response in water cultures particularly with reference to trends in accumulation of potassium.

The authors are pleased to acknowledge the analytical assistance of Dr. Maria Kolisch.

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A STUDY OF THE PHYSIOLOGICAL EFFECTS OF ELEMENTAL SULPHUR DUST ON CITRUS FRUITS[1]

FRANKLIN M. TURRELL

(WITH NINETEEN FIGURES)

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Introduction

Elemental sulphur was referred to as a disinfectant by Homer nearly a thousand years before Christ (**71**), but was probably first used for pest control in agriculture by Robertson in 1824 (**27**). In the years that followed, many improvements were made in increasing the toxicity of sulphur. Lime was added; lime and sulphur were boiled, and then self-boiled; bordeaux was added to sulphur; colloidal sulphur, wettable sulphur, flotation sulphur, and micronized sulphur were then used, and later dithiocarbamates; other sulphur-containing organic compounds followed in succession (**27**).

Sulphur, in various forms, is now used specifically for control of certain pests. It is also used generally as a diluent, and is entered as a component in programs where more specific control measures have not been worked out. One of its chief shortcomings as an insecticide or fungicide is its tendency to "burn" leaves and fruit during periods of hot weather. This characteristic has been especially refractory to field research studies, and its control has lagged in comparison with the enhancement in insecticidal and fungicidal properties of sulphur. The present work on the physiology of injury to citrus fruit by elemental sulphur dust was initiated to lay a basis for the development of sulphur insecticides and fungicides having lower potentially injurious effects during hot weather.

Sulphur, in one form or another, has been the subject of numerous investigations in both animal and plant physiology over a period of many years. The extensive amount of work done in this country and abroad on the fungicidal action of sulphurs has been reviewed recently by FREAR (**14**), HORSFALL (**27**), and ROBERTS (**56**). WILCOXON and MCCALLAN (**86**) have found that "the most important single factor determining the toxicity of sulphur dust is the number of particles furnished by unit weight of the material." As the insecticidal and fungicidal effectiveness has increased, injury to plants has also increased. It seems probable, therefore, that the two problems are similar, at least at certain points.

Symptoms of injury by elemental sulphur

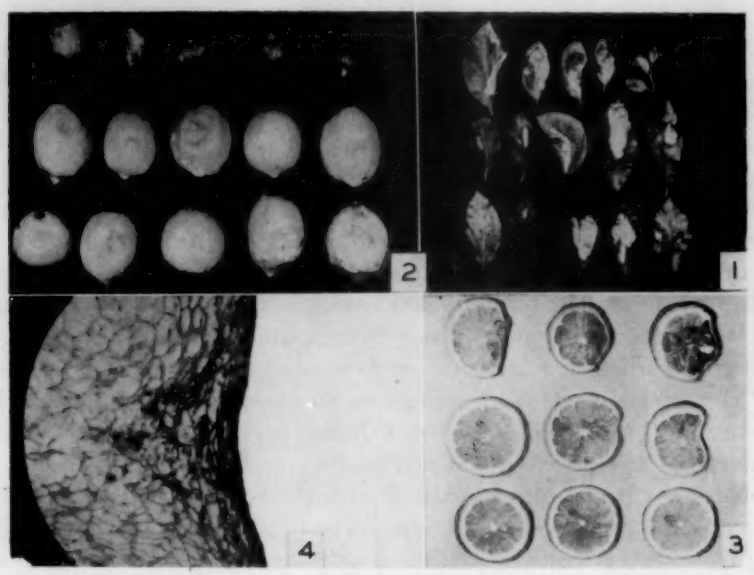
In the humid coastal areas of California both fruit and foliage may be injured on sulphur-dusted citrus trees during hot weather. In the drier inland regions it is usually the fruit alone that is injured. Injury is con-

[1] Paper no. 612, University of California Citrus Experiment Station, Riverside, California.

fined to those parts of the leaves and fruit that are exposed to the direct rays of the sun.

Leaf injury is characterized by a necrosis ranging from small spots to extensive areas which may include the greater part of the chlorenchyma (fig. 1).

Fruit injury in yellow lemon fruit is characterized by small, white translucent spots. Frequently the affected area becomes yellowish pink. In ripe navel oranges a green spot may appear in the injured area. In green



FIGS. 1–4. 1. A type of sulphur injury or "burn" on Eureka lemon leaves. Maximum air temperature at time of injury, 103° F. 2. Sulphur injury or "burn" on green and yellow Eureka lemons. Maximum air temperature at time of injury, 103° F. 3. Sections through sulphur-injured ("burned") Eureka lemons, showing severe injury (two upper rows) and normal, uninjured sections (bottom row). 4. Photomicrograph through a small, sulphur-injured ("burned") spot in peel of Eureka lemon (radial section, ×110). Note the dark-colored, safranin-stained tissue at the surface of the peel, the collapse of cells at the surface, and the coagulation of protoplasm in noncollapsed cells in radial axis of "burn."

lemon fruit destruction of the chlorophyll leaves small to large yellowish-pink to light-red areas. These are occasionally surrounded by a small green ring, which is in turn surrounded by a yellow ring. In green navel oranges the center of the injured area may be light green in color, surrounded by a pinkish-brown ring. About two days after injury, the severely affected areas on both ripe and green fruits becomes sunken, brown, and hardened (fig. 2). The oil gland protrudes prominently from the collapsed and sunken tissues surrounding these areas. Similar markings may be produced with an infra-red lamp. The injury may be superficial, affecting only the outer surfaces of the peel, or it may also involve the pulp (fig. 3).

Histological sections through necrotic areas indicated cellular collapse and coagulation of the proteinaceous components of the cytoplasm. The collapsed areas stained very deeply with safranin, in contrast to surrounding uninjured tissue. This suggests a more acid reaction in the injured cells (fig. 4). In a few sections of fruit treated with sulphur in oil, where stomata were visible, the tissue underlying the stomata also stained red, indicating that penetration of the tissue by sulphur in oil may be through the stomata.

Experimental procedure and results

Penetration of fruit epidermis by elemental sulphur

Elemental sulphur is insoluble in water and has a low vapor pressure at terrestrial air temperatures. It has chemical properties much like oxygen, and combines readily with many elements. It is readily oxidized or reduced by many compounds and elements and in such form may be readily soluble in water, forming weak to strong acids. It volatilizes slowly at terrestrial air temperature in the form of S_8 (**13**). Volatility increases rapidly with temperature (**13, 17, 36, 75**), the rate increasing logarithmically as the temperature increases (fig. 5).

While it is generally known that elemental sulphur slowly oxidizes in air, and in the presence of dew forms dilute sulphur acids on citrus fruits, penetration of these acids into the fruit through the resin-plugged fruit stomata is unlikely, since the stomatal structure is particularly resistant to penetration of water (**77, 79**). The thick cuticle of 4 to 6 μ also probably prevents the rapid entrance of such acid solutions except where it may have been broken by injury.

The coincidence of fruit injury and higher volatility of sulphur during periods of high temperatures suggests the penetration of the fruit epidermis by sulphur. That such penetration does take place was indicated by some simple experiments in which lemon halves from which the pulp had been removed were sealed to stender dishes containing a little 2% zinc acetate solution. The outer portion of the peel was then dusted with elemental 325-mesh, 99.98% pure sulphur, and the halves were incubated $2\frac{1}{2}$ to $6\frac{1}{2}$ hours at temperatures ranging from 90° to 115° F. Subsequently, the zinc sulphide in the zinc acetate solution was converted to methylene blue by standard methods (**12**). The results recorded (table I) show that increasing amounts of H_2S were absorbed by the zinc acetate solution with increasing temperatures.

H_2S was assayed in the foregoing experiment, but since the H_2S might have been formed from the action of sulphur on the fruit cuticle, proof of penetration of the fruit epidermis by sulphur was not adequate. Consequently, radioactive sulphur as S^{35} was applied as a dust to fruits which were subsequently incubated for 6 hours at 140° F in gas-tight jars. After such treatment the fruit surface was carefully cleaned, and counts of the disintegration of S-atoms were made with a Geiger counter on fresh tan-

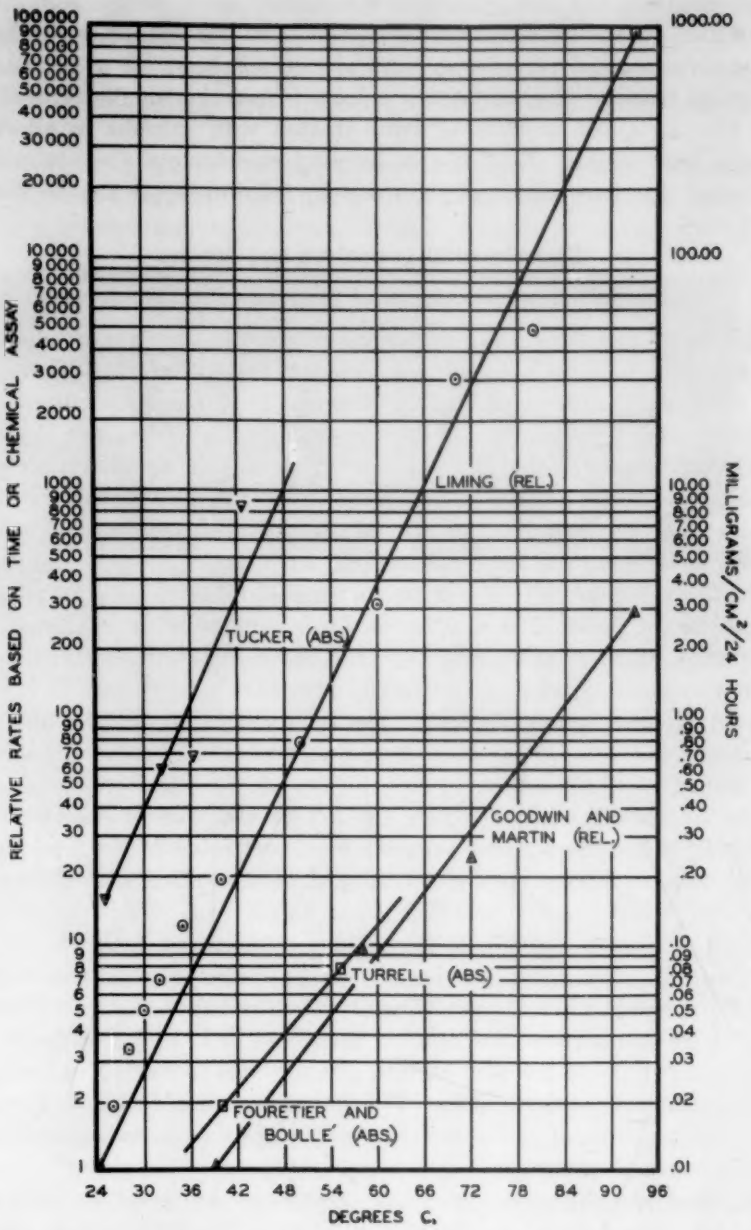


FIG. 5. Semilogarithmic plotting of rates of vaporization of elemental sulphur, as determined by various workers. Data in terms of absolute rates, in milligrams/cm.²/24 hours (right ordinate), are from FOURETIER and BOULLÉ (13), TUCKER (75), and TURRELL; those in terms of relative rates (left ordinate) are from GOODWIN and MARTIN (17) and LIMING (36).

gential sections taken at varying depths in the peel. The results shown (table II) indicate that considerable radioactive sulphur had penetrated the fruit peel to a depth of at least 240 μ, and possibly a small amount to a depth of 480 μ. This detectable degree of penetration (240 μ) corresponds roughly to the depth of injury often observed under field conditions,

TABLE I

PENETRATION OF LEMON PEEL BY ELEMENTAL SULPHUR, AS INDICATED BY H_2S PRODUCTION WHEN HOLLOWED-OUT, SULPHUR-DUSTED FRUIT HALVES WERE INCUBATED THREE OR SIX HOURS AT VARIOUS TEMPERATURES

Temperature	H_2S per lemon half after incubation			
	Three Hours		Six Hours	
	Non-Dusted	S-Dusted	Non-Dusted	S-Dusted
° F	μg.	μg.	μg.	μg.
90	0.00	0.50	0.00	0.00
95	0.00	.....	.....	.....
100	0.00	8.60	0.00	0.30
105	0.00	16.21	0.00	.....
110	0.00	21.59	0.00	24.40
115	0.00	18.52	0.14	21.68

where, after superficial injury of the fruit, the surface layers of the peel crack and scale off, the "scale" often approximating 360 μ in thickness. The superficial injury, however, shown in figure 4, was 115 μ at the deepest point. Apparently, somewhat similar penetration and accumulation of sulphur compounds has been found in the epidermis of animals exposed to mustard gas labeled with radioactive sulphur (**2**).

TABLE II

RADIOACTIVITY OF 0.25-CM.^{2} TANGENTIAL SECTIONS (80 μ THICK) OF PEEL OF LEMON FRUIT DUSTED WITH S^{35} AND INCUBATED FOR SIX HOURS AT 140° F

Distance of section from external surface of peel	Counts/second above background in	
	Nondusted peel	S^{35}-dusted peel
μ		
0	−0.003	+0.025
240	−0.034	+0.148
400	+0.008	+0.010
480	0.000	+0.011
560	+0.016	−0.005

Solid elemental sulphur particles which pass through a 325-mesh screen average 16.7 μ in diameter (**29**), and 35% of some ground sulphurs, are 10 μ or less (**16**). These particles are much too large to enter stomatal pores which are $7 \times 3\mu$ in cross-sectional dimensions and plugged with resin so that the entrance channels are about 0.5 μ (**77**).

Experiments using glass slides waxed with orange wax, sulphur-dusted, produced only very small amounts of H_2S when incubated at 105° F.

This result indicates that the reaction of sulphur with fruit to produce H_2S is due, not to the waxy surface of the fruit, but either to the penetration of the rind beyond the cuticle surface or to a reaction with a substance exuded by the peel. Since elemental sulphur has been shown to act at a distance through a collodion membrane and through an air space (**40**), this point was not reinvestigated. The positive correlation between H_2S formed and temperature, the penetration of radioactive sulphur, and the morphological evidence indicate that elemental sulphur may enter the fruit peel as a gas, but in view of recent findings, it appears that judgment should still be reserved on the mode of entrance of sulphur. UMBREIT *et al.* (**81**) state that, in *Thiobacillus thiooxidans*, sulphur dissolution occurs in the fat globule at the cell terminus. ROBERTS *et al.* (**55**) have found that the cuticle of apple leaves is a discontinuous layer. SCOTT and BAKER (**59**) have shown that wax channels penetrate through the outer wall of the epidermis in Washington Navel oranges, and MORELAND and ANDERSON (**45**) have made similar observations on *Aucuba japonica* leaves. Although radioactive elemental sulphur vapor has been shown by TURRELL and CHERVENAK (**78**) to give radioactive products in the peel of lemons, this does not exclude the possibility that the S^* vapor may have first dissolved in the wax and penetrated in such form through the wax channels.

PRODUCTION OF H_2S GAS BY ELEMENTAL SULPHUR

When Washington Navel and Valencia oranges (**80**) and Eureka and Lisbon lemons were dusted with pure elemental 325-mesh sulphur (fig. 6) and placed in gas-tight containers provided with gas-washing and gas-ab-

FIG. 6. Photomicrograph showing typical deposit of 325-mesh sulphur on a Washington Navel orange used in these experiments. (× 120.)

sorbing bottles containing 2% zinc acetate (fig. 7), at temperatures of 90° to 140° F, a gas was produced and collected. This gas was identified as H_2S by standard methods, namely by odor, darkening of lead acetate papers, development of methylene blue (**12**), and iodine titration.

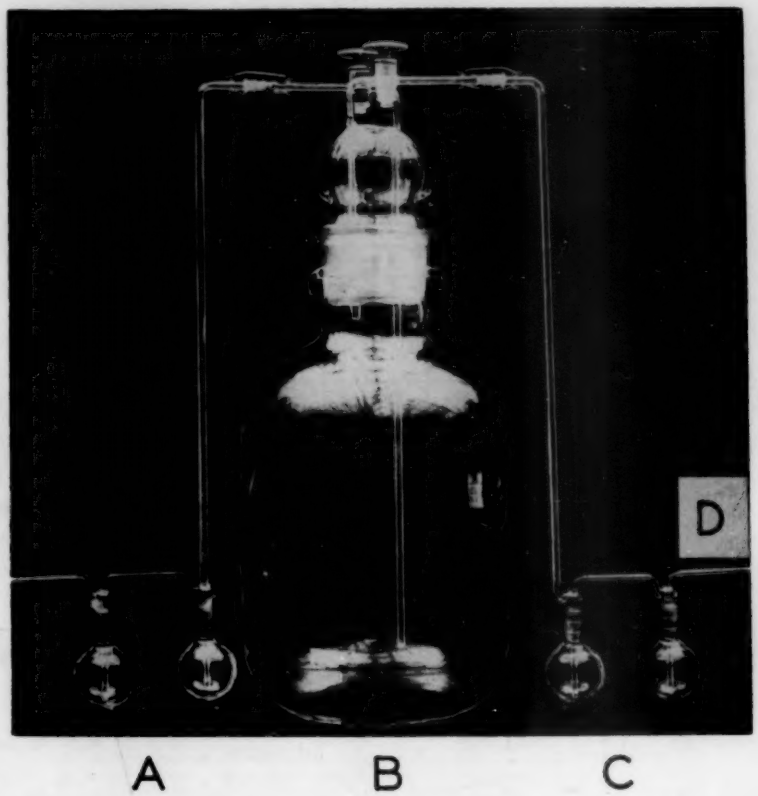


FIG. 7. Apparatus used to collect the gases formed by sulphur-dusted citrus fruit: Pyrex-bottle reacting chamber (B) in which 28 sulphur-dusted citrus fruits could be incubated, air-washing (A) and gas-absorption (C) bottles. All joints were ground glass. Bottles in A and C contained 2% zinc acetate, or a modification of this solution, when H_2S only was absorbed. When both SO_2 and H_2S were absorbed, the bottle on the left in A and in C contained phosphate-borate buffer instead of zinc acetate. Vacuum was applied at D. Temperature was maintained constant (to ± 0.5 F) by immersing the apparatus in a thermostat.

In each of 110 experiments, air that was washed free of H_2S (fig. 7A) was passed over 28 sulphur-dusted Eureka lemons in a bottle (fig. 7B), maintained at various specified temperatures in a thermostat, for 2-day periods. The H_2S evolved in each of these systems was collected in 2% zinc acetate solutions (fig. 7C) and assayed by the Almy method (**1**), except that a photoelectric colorimeter was used to determine methylene blue concentration. It was found that a small amount of H_2S was produced at

90° F, and that increasing amounts were produced at increasing temperatures, up to 110° (fig. 8). The total H_2S produced at 115° was about equal to that produced at 110°, but as the temperature increased, up to 135°, the total amounts of gas produced decreased (see following section). The

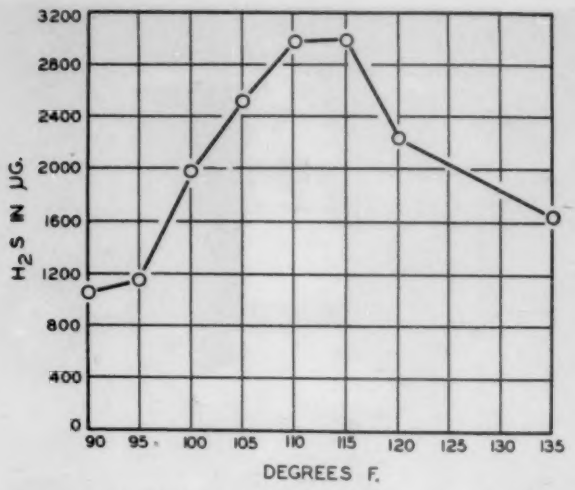


FIG. 8. Average total H_2S production per sample of 28 sulphur-dusted Eureka lemons incubated at various temperatures until tissue softened (2½ days), with H_2S absorption and without SO_2 absorption. Nondusted controls produced no H_2S.

TABLE III

AVERAGE H_2S PRODUCTION OF SAMPLES CONSISTING OF 28 SULPHUR-DUSTED LEMONS INCUBATED AT VARIOUS TEMPERATURES

TEMPERATURE	TOTAL H_2S	STANDARD DEVIATION	H_2S PER HOUR
° F	µg.		µg.
90	1059	.....	8.97
95	1309	916.1	9.02
100	1789	1114.1	12.84
105	2522	1609.3	37.93
110	2973	2174.6	33.40
115	2974	1638.6	30.78
120	2241	1328.2	22.66
125	.....	.....	.....
130	.....	.....	.....
135	1672	580.0	64.31

standard deviations for these determinations were extremely large (table III), and it was necessary to make a large number of analyses in order to show statistical significance between the means. The decreases in the total amounts of H_2S produced with increasing temperatures above 115° F are significant, as indicated by the analysis of variance in table IV.

The rate of H_2S production increased with temperature up to 105° F, then dropped off gradually at increasing temperatures up to 120°. How-

ever, the rate was most rapid at 135° (table III). The greatly accelerated rate of H_2S production at 135° was accompanied by the breakdown of the fruit after 24 hours of incubation. Possibly enzyme inactivation occurred at this high temperature and reduced the total H_2S produced. LUCAS and

TABLE IV

ANALYSIS OF VARIANCE OF THE AVERAGE TOTAL AMOUNTS OF H_2S PRODUCED BY SAMPLES CONSISTING OF 28 SULPHUR-DUSTED EUREKA LEMONS AT INCREASING INCUBATION TEMPERATURES (115° TO 135° F)

SOURCE OF VARIATION	D.F.	SUM OF SQUARES	MEAN SQUARE
Total	31	44,712,777	1,442,348
Between temperatures	2	9,137,036	4,568,518*
Within temperatures	29	35,575,741	1,226,750

* F required for odds of 5 in 100 = 3.33; F found = 3.72.

KING (**39**) showed a similar trend in the oxidation of cysteine by iodine. Further investigation was carried on in order to find the factors involved in the lowering of total H_2S produced at elevated temperatures (see discussion below).

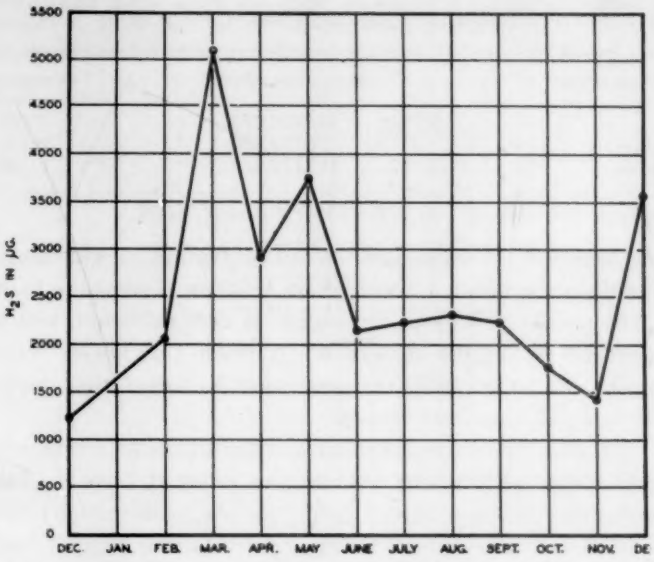


FIG. 9. Average total H_2S production (by months) per sample of 28 sulphur-dusted Eureka lemons picked twice a week for a period of a year and incubated at 105° F until tissue softened (two days), with H_2S absorption and without SO_2 absorption. Nondusted controls produced no H_2S.

In addition to the temperature sensitivity of this reaction, there appears to be a seasonal periodicity. Observations on the H_2S produced by samples of 28 sulphur-dusted Eureka lemon fruits collected twice a week from the same grove at the Citrus Experiment Station for a period of one year

and incubated 2½ days (until the tissue softened) at 105° F are shown in figure 9. The maximum H_2S production occurring in the winter and spring months was significantly different statistically (table V) from the low production which occurred during the summer and fall months, and coincides with observations by growers, that lemon fruit are more susceptible to injury during periods of hot weather in spring months than in summer months. This relative sensitiveness of the fruit during the spring months may be indicative of a higher glutathione content in the spring.

The variation in H_2S production of citrus fruit at different seasons roughly corresponds to the percentages of sulphur found by MARSH (**43**) for the young bark of Wealthy and Payne Late Keeper apple trees, indicating that there may be a correlation between the glutathione content of plants and seasonal variation in H_2S production. In general, the percentage of sulphur in bark was high in spring, low in summer, and high in winter. Citing the work of Abbott and of Mitra, Marsh shows a rough

TABLE V

ANALYSIS OF VARIANCE OF THE AVERAGE TOTAL AMOUNTS OF H_2S PRODUCED BY SAMPLES CONSISTING OF 28 SULPHUR-DUSTED EUREKA LEMONS TREATED AT VARIOUS INTERVALS FROM DECEMBER TO MAY AND FROM JUNE THROUGH NOVEMBER, AND INCUBATED AT 105° F

SOURCE OF VARIATION	D.F.	SUM OF SQUARES	MEAN SQUARE
Total	56	143,356,285	2,559,934
Between seasons	1	26,178,409	26,178,409*
Within seasons	55	117,177,876	2,130,507

* F required for odds of 1 in 100 = 7.17; F found = 12.29.

parallelism between the hydrogen-ion concentration of non-bearing apple spurs and sulphur content. The highest sulphur percentage occurs in the middle of the period of highest hydrogen-ion concentration, and the lowest sulphur percentage occurs coincidentally with the lowest hydrogen-ion concentration. SLUITER (**65**) demonstrated a correlation between H_2S production and pH in animal tissues.

IVANOV (**30, 31**) has shown that winter hardiness in citrus is inversely proportional to the glutathione content (in reduced form). The physiological similarities between injury to citrus (*i.e.*, desiccation) from freezing and from elemental sulphur during warm weather suggest that we may be dealing with similar factors.

No measurable quantity of H_2S was obtained from the non-dusted control fruit in these experiments, the presence of elemental sulphur appearing to be essential to H_2S production.

PRODUCTION OF SO_2 GAS BY ELEMENTAL SULPHUR

It has been shown (**72**) that plant materials yield SO_2 as well as H_2S during alkaline hydrolysis. Therefore it seemed advisable to investigate the production of SO_2 by citrus fruit treated with elemental sulphur dust.

An SO_2-absorption bottle containing a phosphate-borate buffer similar to that used by THOMAS *et al.* (**72**) was consequently inserted ahead of the H_2S-absorption bottles in both the air-washing train and the gas-absorption train (fig. 7). Assay of SO_2 was made by titration of the phosphate-borate buffer with standard iodine.

Table VI shows the results of the assays for SO_2 collected from 28 lemons that had been sulphur-dusted and then incubated for 2-day periods

TABLE VI

AVERAGE SO_2 PRODUCTION OF SAMPLES CONSISTING OF 28 SULPHUR-DUSTED LEMONS INCUBATED AT VARIOUS TEMPERATURES

TEMPERATURE	SO_2	
	PER HOUR	TOTAL
° F	μg.	μg.
90	0.21	9.89
105	4.38	199.64
120	6.70	364.07

at various temperatures as the gases were continuously drawn through the absorption train. In these experiments both the total H_2S and the total SO_2 recovered increased almost linearly with increase in temperature up to 120° F (fig. 10). Probably the apparent reduction in the total

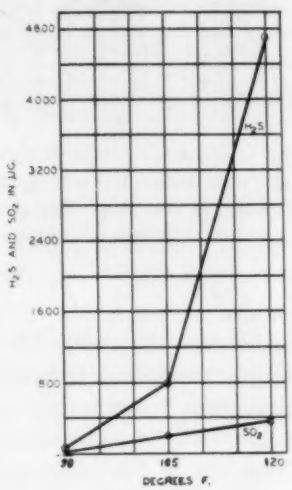


FIG. 10. Average total H_2S and SO_2 production obtained simultaneously per sample of 28 sulphur-dusted Eureka lemons, on incubation at various temperatures for 2-day periods. Nondusted, incubated controls produced no H_2S or SO_2.

amount of H_2S produced at high temperatures in the original experiments in which H_2S only was collected and assayed by the Almy method (see page 19), was due to the decolorizing action of SO_2 on the methylene blue formed by H_2S. It likewise follows that either less SO_2 was released by

lemon fruit in spring and fall than at other times of the year or the values for the total H_2S produced were actually higher than those found. The H_2S production in various seasons was statistically significant, however, as shown in table V.

In the experiments described above, the solutions in the gas-washing and gas-absorption bottles were assayed morning and evening and replaced with new solutions, and the duration of each experiment was ordinarily 2 to 2½ days. In the experiments presently to be described it was desirable to obtain the rate of H_2S and SO_2 production hourly. To this end the gas-absorption bottles were removed each hour during the day. The experiments were then allowed to run through each night without change of the solutions in the absorption bottles until the following morning.

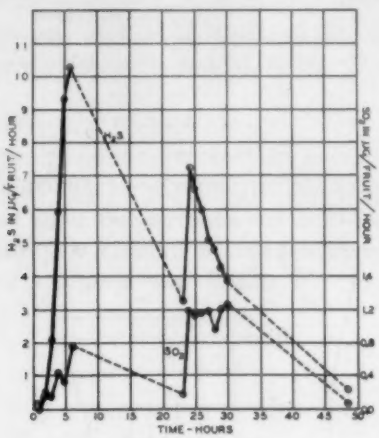


FIG. 11. Average rate of production of H_2S and SO_2 per sulphur-dusted Eureka lemon, per hour during the day (solid lines) and over a 17-hour period at night (dashed lines). Fruit incubated at 120° F, in the dark, throughout the experiment. H_2S and SO_2 absorbed simultaneously. Nondusted, incubated controls produced neither H_2S nor SO_2.

One of the notable changes evident in the results after the introduction of the SO_2-absorption bottles was the reduction in the variability of the H_2S production at the lower incubation temperatures. Without SO_2-absorption bottles, the H_2S standard deviation was 916 at 95° F, 1609 at 105° and 1328 at 120° (table III); with the SO_2-absorption bottles, the standard deviation was 28 at 90° and 316 at 105°, but at 120° the standard deviations jumped to 2011. In the higher temperature ranges of the present experiments (*i.e.*, above 115° F), another factor may have been operating in addition to SO_2 production, such as NH_3 formation due to the hydrolysis of glutamine. This may have increased the variability of determinations of H_2S over that where no SO_2 absorber was used.

It will be noted in figure 11 that the H_2S produced by each Eureka lemon per hour increased very rapidly during the first six hours of incuba-

tion at 120° F, during the day, when the gas-absorption bottles were changed hourly. After the first night period of 17 hours, during which time the bottles were not opened, the mean H_2S produced per hour dropped. Inasmuch as the experiments were run in the dark and all factors were the same over the entire experimental period, with the exception of the opening of the bottles, and there was 370 times as much zinc acetate solution as required to absorb the largest amount of H_2S obtained, it appears that the opening of the bottles was responsible for the increase in the rate of H_2S production. This suggests that the oxygen in the air may be important in increasing H_2S production either directly or indirectly.

The SO_2 curve (fig. 11) shows somewhat similar results, although the curve is a little more irregular than the H_2S curve. In most of the experiments no correspondence was noted between the amount of gases produced and the quantity of sulphur dust applied to the fruits. Similar results were noted by NORRISH and RIDEAL (**47**) in their *in vitro* studies on the conditions of the direct union of hydrogen and sulphur at temperatures of 230° to 360° C. They also found that 3% oxygen catalyzed the reaction between sulphur and hydrogen to give H_2S (increased the rate about six times), that SO_2 was formed in the reaction bulb along with the H_2S (**48**), and that the rate of SO_2 formation was proportional to the O_2 pressure.

The question may be raised as to whether factors similar to those found by Norrish and Rideal operated in the present experiments. The respiration rate of Eureka lemons was calculated from the formula

$$\log (CO_2)_t = \log (CO_2)_{t=0} + a\,t,$$

derived by GORE (**18**). If $(CO_2)_t = y$, and $(CO_2)_{t=0} = y_0$, then $\log y = \log y_0 + a\,t$, where the CO_2 is given in milligrams per kilogram per hour; t is temperature in degrees Centigrade, and a is a constant determined by Gore. With his constants, $\log y_0 = 0.27$ and $a = 0.034$, the calculated respiration rate at 120° F was 87.28 mg. CO_2 per kilogram per hour, or 0.262 gm. of CO_2 per hour for 3 kg. of fruit. If the respiratory quotient is assumed to be unity, the oxygen requirements would be 0.191 gm. per hour or 3.24 gm. in 17 hours.

The maximum rate of air flow through the apparatus used in the present experiments was estimated to be about 12.8 ml. per minute, supplying about 0.125 gm. of O_2 per 17 hours. The 27 liters of air in the reaction bottle after loss of 2 liters because of expansion on heating would yield about 6.01 gm. of O_2 for respiratory use, and this plus 0.13 gm. of O_2 supplied by aeration would make 6.14 gm. of O_2 available for respiration during the 17-hour incubation period. At the end of the experimental period, 2.90 gm. of O_2, or 47.3%, probably remained, the oxygen used for SO_2 formation (as assayed) being negligible (0.000226 gm.). Thus the normal oxygen content of the air in the bottle dropped from an estimated 20% to 9.5% during the incubation period.

NORRISH and RIDEAL (**49**) found the velocity of SO_2 formation by

direct union at 6.9% and 20.7% atm. O_2 to be respectively 15.72 and 56.7 gm./sec $\times 10^8$, at 245° C. The velocity of SO_2 formation at 120° F in Eureka lemon fruit, at about 9.5% O_2, was 0.162 μg./hour (peak); and at 20% O_2, 0.583 μg./hour (average). For the Norrish and Rideal system the velocity ratio at the two pressures was 0.277; for the present organic system it was 0.278.

Phosphate-borate solution was also adequate. It was calculated that it would absorb more than 1,000 times the SO_2 developed, and that the zinc acetate solution, as mentioned before, could react with 370 times the H_2S produced in any of the present experiments. Since THOMAS (**72**) found that similar solutions effectively separated and absorbed many times the SO_2 and H_2S produced in the present experiments, and since no H_2S was found to have been absorbed in the second trap when double traps were employed (see fig. 7), it seems reasonable to suppose that the saturation of the absorption solutions was not the limiting factor.

If the velocities of the surface reaction of H_2S formation in gm./sec. $\times 10^8$, as given by NORRISH and RIDEAL (**47**) for 265° C at 3% and 1% atm. O_2 (5.84 and 3.00, respectively), are compared with the velocities of H_2S formation in μg./hour in the present experiments at 120° F (fig. 11) for 20% O_2 (10.25, peak) and 9.5% O_2 (3.25, average), we find that $\frac{5.84}{3.00} = 1.95$ and $\frac{10.25}{3.25} = 3.15$. Though the experimental conditions are not equivalent, the evolution of H_2S in the organic system is more rapid than in the physical system. In experiments in which 25 sulphur-dusted test tubes having a surface of 3375 cm.^{2} were used to replace citrus fruit in the incubation experiments, no H_2S or SO_2 was obtained. This suggests that we are working with an enzyme system rather than with physical catalysis.

It is apparent from the present experiments that H_2S and SO_2 may be produced under anaerobic conditions, though in lesser amounts than under aerobic conditions. Our results are in accord with the results of SMYTHE (**67**), who found in his work with extracts of rat liver that the cysteine-cystine system produced H_2S anaerobically.

PRODUCTION OF SO_4 BY ELEMENTAL SULPHUR

Analysis of the dried peel of sulphur-dusted Valencia oranges which had been incubated in closed containers for various periods of time after sulphur-dusting, ranging from 6 to 118 hours, showed an irregular but definite increase in the percentage of sulphur, determined as sulphate by standard methods (**38**), as the length of the incubation period was increased (table VII).

These data, when plotted, show considerable dispersion from the mean curve. This dispersion was due partly to the fact that 65 different samples of fruit were required in order to obtain the 65 different values in the table. The samples were picked at different seasons and may have been

influenced also by the GSH content of the fruit, as indicated for H_2S production. However, the curves appear to rise sharply from the origin and thus indicate a rapid increase in sulphate-sulphur during the first 30 hours of incubation. They then flatten off considerably, with longer incubation periods, at both 105° and 110° F, but decline after 30 hours at 115° F, showing the same general trend as the 17-hour night incubation curves of H_2S and SO_2. The rate of increase in sulphate appears to follow the temperature curve for the surface of the peel. The rate of water loss may also be partly responsible for the apparent increase in the percentage of sulphur, as can be seen by the controls. Nevertheless, comparison of the differences between the percentage of sulphur in the peel of nondusted fruit and of sulphur-dusted fruit shows that the sulphur-dusted fruit has

TABLE VII

PERCENTAGE OF SULPHUR (DETERMINED AS SO_4) IN DRY PEEL OF SULPHUR-DUSTED VALENCIA ORANGES INCUBATED AT VARIOUS TEMPERATURES FOR VARIOUS LENGTHS OF TIME

INCUBATION PERIOD	S IN PEEL OF FRUIT INCUBATED AT								
	105° F			110° F			115° F		
	CONTROL (NONDUSTED)	HEAVILY DUSTED	LIGHTLY DUSTED	CONTROL (NONDUSTED)	HEAVILY DUSTED	LIGHTLY DUSTED	CONTROL (NONDUSTED)	HEAVILY DUSTED	LIGHTLY DUSTED
Hours	%	%	%	%	%	%	%	%	%
6	.....	.....	.....	0.0650	0.0849	0.0711	.....	.....	.....
16	0.0468	0.0713	0.0850	0.0911	0.1004	0.0878	0.0793	0.0916	0.0815
21	0.0647	0.0847	0.0816	0.0960	0.0957	0.0960	0.0817	0.1078	0.0877
29	0.0678	0.0830	0.0778	0.0692	0.0971	0.0959	0.0804	0.1010	0.0883
40	0.0692	0.0861	0.0840	0.0848	0.1080	0.0885	.....	.....	.....
48	0.0819	0.0898	0.0823	0.0716	0.1220	0.0811	.....	.....	0.0958
65	0.0437	0.0654	0.0706	.....	.....	.....	.....	.....	.....
74	0.0654	0.0850	0.0802	.....	.....	.....	.....	.....	.....
87	.....	0.0957	0.0885	.....	.....	.....	.....	.....	.....
93	0.0836	0.0737	0.0857	0.0865	0.1147	0.0848	0.0603	.....	0.0800
118	0.0885	0.0871	0.0812	0.0843	0.1214	0.0892	.....	.....	.....

the higher percentage of sulphur. In the heavily dusted fruit, the percentage of sulphur determined as SO_4 on dry peel appears to increase with temperature (table VIII). Inasmuch as the analyses of the expressed tissue fluids did not show the same general trend with increase in temperature, it may be inferred that the sulphate was not all in solution in the cell tissue fluids as SO_4^{--}, but may have been derived from sulphur-bearing organic molecules such as cysteic acid or sulphenic and sulphinic acids. As in the analyses of dry peel, however, SO_4^{--} content of the peel-tissue fluid of sulphur-dusted fruit was higher than the nondusted, incubated controls (table VIII).

The conversion of elemental sulphur to sulphate ion, furthermore, appears to occur in the tissue fluids. For instance, as shown in table IX, 10 ml. of expressed tissue fluid from the peel of sulphur-dusted, incubated

TABLE VIII

PERCENTAGE OF SULPHUR (DETERMINED AS SO_4) IN PEEL OF SULPHUR-DUSTED VALENCIA ORANGES INCUBATED ON AVERAGE OF 43 HOURS AT VARIOUS TEMPERATURES

TEMPERATURE	CONTROL FRUIT (NON-DUSTED) (C)	FRUIT HEAVILY DUSTED (H)	FRUIT LIGHTLY DUSTED (L)	DIFFERENCE	
				H-C	L-C
	S IN DRY PEEL				
°F	%	%	%	%	%
100	0.0802	.....	0.1029	.....	0.0227
105	0.0699	0.0822	0.0819	0.0123	0.0120
110	0.0787	0.0992	0.0857	0.0205	0.0070
115	0.0754	0.1001	0.0866	0.0247	0.0112
120	0.0603	.....	0.0800	.....	0.0197
	S IN EXPRESSED PEEL-TISSUE FLUID*				
°F	*mg./10 ml.*	*mg./10 ml.*	*mg./10 ml.*	*mg./10 ml.*	*mg./10 ml.*
105	1.92	2.44	2.29	0.52	0.37
110	2.01	2.16	2.36	0.15	0.35

* Method of Gortner and Hoffman (19).

fruit of Eureka lemon assayed 0.37 mg. more sulphur as sulphate than the tissue fluid from nondusted fruit. When a 10-ml. aliquot of expressed sap from the nondusted peel was filtered through 1 gm. of elemental sulphur, it assayed 0.16 mg. more sulphur as sulphate than a similar aliquot not so treated. Similar treatment of 10-ml. aliquots of expressed sap from the sulphur-dusted fruit showed an average increase of 0.12 mg. of sulphur assayed as sulphate. Assay of the sulphur as sulphate in 10 ml. of distilled water that had passed through 1 gm. of sulphur showed a gain of only 0.0174 mg. sulphur over the sulphate-sulphur in distilled-water controls. It seems reasonable to conclude, therefore, that there is a mechanism in the

TABLE IX

SULPHUR (DETERMINED AS SO_4) IN EXPRESSED PEEL-TISSUE FLUID OF NONDUSTED AND SULPHUR-DUSTED EUREKA LEMONS INCUBATED AT 105° F FOR 48 HOURS IN GAS-TIGHT CONTAINERS; DETERMINED ON 10-ML. ALIQUOTS BEFORE AND AFTER FILTRATION THROUGH 1 GM. 325-MESH 99.6% PURE GROUND ELEMENTAL SULPHUR

S IN EXPRESSED PEEL-TISSUE FLUID			
NONDUSTED FRUIT		S-DUSTED FRUIT	
BEFORE FILTRATION	AFTER FILTRATION	BEFORE FILTRATION	AFTER FILTRATION
mg./10 ml.	*mg./10 ml.*	*mg./10 ml.*	*mg./10 ml.*
0.96	1.18	1.40	1.58
1.14	1.23	1.43	1.50
Av. 1.05	1.21	1.42	1.54

cell sap which converts elemental sulphur to sulphate, and that its reaction with elemental sulphur (at 105° F, for 48 hours) does not destroy its activity, though it appears to reduce it slightly. A somewhat analogous situation was found by PIRIE (**53**), who incubated slices of rat liver with thiosulphate and obtained SO_4.

It might be argued that the conditions to which we have subjected fruit in the laboratory, namely, the water-vapor-saturated air and the enclosure of the escaping gases or rapid withdrawal of them, are not similar to conditions in the field; and that the conversion of elemental sulphur to sulphate probably does not occur in the field. Analysis of peel of the burned and unburned sides of commercially sulphur-dusted lemons and oranges which were on the trees in the grove at the time of injury, showed that the burned sides of the peel contained higher percentages of sulphur, when determined as sulphate on dry peel, than did the unburned sides (table X).

TABLE X

PERCENTAGE OF SULPHUR (DETERMINED AS SO_4) IN DRY PEEL OF "BURNED" AND "UNBURNED" SIDES OF SAME CITRUS FRUITS, COMMERCIALLY SULPHUR-DUSTED AND ON TREES AT TIME OF INJURY

KIND OF FRUIT	S IN DRY PEEL	
	"UNBURNED"	"BURNED"
	%	%
Lemons (large yellow)	0.0742	0.0817
Lemons (small green)	0.0654	0.0738
Valencia oranges	0.0638	0.1011
Valencia oranges	0.0649	0.1019

LOWERING OF pH BY ELEMENTAL SULPHUR

The higher percentages of SO_4 in the tissue fluid of sulphur-treated fruit peel, as compared with that of the nondusted fruit, suggest that the sulphate is present in soluble form. The buffer capacity of tissue fluids of citrus fruit peel is slight (**64**), and the increase in SO_4, though small, might be sufficient to change the pH, if present in the form of H_2SO_4.

Determinations of pH were made with a glass electrode on 25 gm. of peel macerated in 100 ml. of water in a Waring blendor, after the resulting brei was centrifuged, and filtered through cotton. The pH values presented in table XI show that the peel of the nondusted fruit incubated for only 3 hours at 140° F, became more acid than that of the nondusted, nonincubated controls. The peel of sulphur-dusted, incubated fruit became even more acid, however, than that of the nondusted, incubated fruit. The apparent decrease in pH of the incubated controls was due in part to partial dehydration during incubation, in part possibly to an accumulation of organic acids, and in part possibly to the oxidation of cystine. According to WOOD (**88**), "loss of water by tissues also results in decrease in protein-S

and increase in SO_4-S. It is generally assumed that this oxidation proceeds by way of cystine."

The influence of temperature of incubation of nondusted and sulphur-dusted fruit on the pH of water extracts of the peel is shown in table XII. At temperatures of 86° to 104° F there appears to be no large decrease of pH in either nondusted or sulphur-dusted fruit. But at 113° and 122° the

TABLE XI

AVERAGE PH OF WATER EXTRACTS OF PEEL OF NONDUSTED AND SULPHUR-DUSTED CITRUS FRUITS INCUBATED FOR THREE HOURS AT 140° F (EXTRACTION DILUTION 1: 4)

KIND OF FRUIT	AVERAGE PH OF PEEL EXTRACT*		
	NONINCUBATED, NONDUSTED	INCUBATED	
		NONDUSTED	S-DUSTED
Eureka lemon (silver)	5.59	5.16	4.66
Eureka lemon (ripe)	5.29	4.53	4.42
Washington Navel orange	5.74	4.87	4.79

* Analysis of variance indicates that differences in pH between treatments are highly significant (1% level).

pH of the sulphur-dusted peel extract shows a significant decrease below that of the nondusted, while at 131° and 140° the pH is again not significantly lower in the peel extract of sulphur-dusted fruit, as compared with the nondusted. These data are roughly in line with those on percentages of

TABLE XII

AVERAGE PH OF WATER EXTRACTS OF PEEL OF NONDUSTED AND SULPHUR-DUSTED EUREKA LEMONS INCUBATED FOR 48 HOURS AT VARIOUS TEMPERATURES (EXTRACTION DILUTION 1: 4)

TEMPERATURE	AVERAGE PH OF PEEL EXTRACT	
	NONDUSTED	S-DUSTED
°F		
86	4.53	4.51
95	4.61	4.73
104	4.72	4.75
113	4.79	4.22
122	3.82	3.43
131	3.27	3.20
140	2.99	3.01

sulphate found for fruit incubated at different temperatures and for different periods of incubation, as shown in tables VII and VIII.

Apparently, there is an optimum incubation time and temperature for sulphate formation and pH lowering. As will be shown later, two factors may be operating, namely, enzyme inactivation and ammonia formation. This optimum appears to exist at temperatures of approximately 105° to 122° F, and for incubation periods of about 48 hours. Temperatures of 44°

C and 63° C at pH 6.5 are known to cause hydrolysis of glutamine to the extent of 3.2 and 19.0% respectively in two hours (**83**). Incubation for a short period of three hours at the high temperatures of 140° F was shown, however, also to lower the pH significantly. A short exposure of about three hours, in which fruit-peel temperatures of 115° to 140° F are maintained, reproduces fairly well conditions in the field when "fruit-burn" is observed. SESSIONS (**60**) was of the opinion that sulphur injury "to the host plant" was caused by acids produced by oxidation of sulphur, but he gave no data to support his assumptions. This is an important point which is discussed below.

H_2S GAS INCREASES SO_4 AND LOWERS pH

A rather large number of experiments were conducted in which lemons and oranges were incubated at various temperatures in atmospheres containing various concentrations of H_2S gas. Suffice it to say here that fruits were injured by 2½-hour exposure to concentrations as low as 40 parts per million of H_2S gas when the peel temperatures were 104° F, but it was six days after treatment before the injury appeared. Likewise, in experiments in which nondusted fruit was irradiated with an infrared lamp, symptoms of the threshold injury by infrared radiation did not appear for three to six days after irradiation. At higher concentrations of H_2S the peel at first had a translucent, water-soaked appearance, and later became bleached and then brownish. Those who were well acquainted with the symptoms of sulphur-burn in the field agreed that fruit treated with H_2S in the laboratory had the typical appearance of fruit injured in the field.

It has been shown that crystalline elemental sulphur volatilizes to form sulphur vapor; that from elemental sulphur citrus fruits produce H_2S gas, SO_2 gas, and finally SO_4^{--}; and that the pH of the peel sap is lowered. If this chain of reactions is begun, not with elemental sulphur but with H_2S gas, by incubating lemons for various periods in an atmosphere of H_2S in the critical temperature range, SO_2 is produced just as with elemental sulphur. Presumptive evidence that SO_2 was produced was at first indicated by the fact that the H_2S, when withdrawn from the fruit after a period of treatment, often failed to develop methylene blue. Presumably, this was because the SO_2 decolorized the solution. Experiments conducted at 105° F, in which any SO_2 formed from H_2S was absorbed in a phosphate-borate buffer, showed that about five times as much SO_2 was formed from H_2S as from elemental sulphur applied as a dust, *i.e.*, about 0.00143 gm. SO_2 for 28 fruits. Sulphate (0.215% sulphur per gram dry weight of peel) was also formed as a result of incubating citrus fruit for 18 hours at 105° F in an atmosphere of H_2S (1-cm. pressure), as compared with 0.13% sulphur in the incubated controls. For other experiments, the percentage of sulphur determined as sulphate for various incubation periods is shown in table XIII. The SO_4 formed in these experiments appeared to be in the ionic

condition, since it lowered the pH of the peel sap of the H_2S-treated fruit below that of the nontreated, incubated controls (table XIII).

TABLE XIII

AVERAGE PERCENTAGE OF SULPHUR (DETERMINED AS SO_4) IN DRY PEEL OF EUREKA LEMONS INCUBATED AT 105° F IN AN ATMOSPHERE OF H_2S GAS (1-CM. PRESSURE) FOR VARIOUS LENGTHS OF TIME

INCUBATION PERIOD	S IN DRY PEEL		
	INCUBATED, NONTREATED (N)	INCUBATED IN H_2S (T)	T-N
Hours	%	%	%
1	0.0666	0.2469	0.1803
3	0.1048	0.1892	0.0844
4	0.0901	0.1965	0.1064
11	0.1118	0.2260	0.1142
17*	0.1099	0.2230	0.1131

* The pH of the peel extract of this sample was as follows: (N) 4.92, (T) 4.10.

SO_2 GAS INCREASES SO_4 AND LOWERS PH

It has been reported by SCHROEDER and REUSS (**46**) that smoke-injured 1-year-old pine needles contained 0.460% sulphuric acid, as compared with 0.101% for healthy needles of the same age. Presumably, the SO_2 content of the smoke raised the percentage of acid. Likewise, LORENZ (**46**) has shown that an increase in smoke increased the sulphur trioxide in spruce needles from 50 to 90%, and in pine needles from 9 to 20%, over comparable controls.

Since elemental sulphur and H_2S gas both were oxidized to SO_4 in the present experiment, and the pH of the citrus peel tissue fluid was lowered, it was of interest to know whether or not SO_2 gas would be converted by the fruit to SO_4.

When Eureka lemons were placed in an atmosphere containing 16 p.p.m. SO_2 gas and incubated at 105° F, there appeared to be an increase in the SO_4 in the fruit peel after 18 hours' exposure, although the pH was not lowered (table XIV). When the concentration of SO_2 was increased to between 2800 to 3200 p.p.m. and the fruit was incubated in this atmosphere, there was an increase in the SO_4 formed and a lowering of the pH of the tissue fluid (table XIV), indicating acid formation. The evidence from this series of experiments indicates a series of reactions of citrus peel tissue with sulphur and its compounds such as gaseous elemental sulphur, H_2S, or SO_2, which are oxidized to SO_4^{--}. The appearance of the SO_2-injured fruit was also considered similar to that of fruit injured by elemental sulphur dust in the field.

This injury in citrus fruit peel appears to be similar to the injury caused by the oxidation of SO_2 to SO_4, observed in alfalfa leaves by THOMAS and HILL (**73**), and in subsequent work by THOMAS *et al.* (**74**),

who showed that fumigation of alfalfa leaves with SO_2 resulted in a lowering of the pH of the alfalfa leaf tissue fluid 0.3 pH units if visible injury occurred. In SO_2-injured dahlia leaves, these workers observed a lowering of 1.0 pH unit.

Oxidation and reduction of glutathione by elemental sulphur

The production of H_2S by living tissues when treated with elemental sulphur has been observed by numerous investigators working with a wide variety of tissues. Guthrie (**24**), however, pointed out the specificity of the reaction between glutathione (GSH) and elemental sulphur and developed a method of analysis based upon it. Tarver and Schmidt (**70**)

TABLE XIV

The pH of peel extract and average percentage of sulphur (determined as SO_4) in dry peel of Eureka lemons incubated 18 hours at 105° F in atmospheres of various concentrations of SO_2 gas

Experiment No.*	Fruit incubated, nontreated		Fruit incubated in SO_2		
	Sulphur content of peel	Acidity of peel extract	SO_2 concentration	Sulphur content of peel	Acidity of peel extract
	%	*pH*	*p.p.m.*	%	*pH*
I	0.0566	4.99	16	0.0758	5.16
II	0.0798	4.70	3200	0.2554	3.76
III†	0.0864	4.98	3200	0.2070	4.32
IV	0.1207	3.50	2800	0.3048	3.26

* Fruits in experiments I, II, and III were of different maturity; those of experiment IV were from trees on different rootstocks.
† Fruits incubated for 23 hours.

gave experimental proof of this method for thiol compounds, using radioactive sulphur, and for cysteine (RSH), showed that the reaction is

$$2\ RSH + S^* \longrightarrow RSSR + H_2S^*$$

The activity of the H_2S^* evolved accounted for "most of that added," and none of it (*i.e.*, less than 0.5%) appeared in the cystine formed.

In order to establish the possibility of this reaction in lemon fruit, analyses were made of the peel of ripe Eureka lemons, using the Guthrie method (**24**). The results of 26 analyses are shown in table XV. The average glutathione (reduced form) content of the peel was 29.5 mg./100 gm. fresh tissue. Calculation indicates that 28 lemons having 897 gm. of peel would contain 0.264 gm. GSH, which would yield 0.014 gm. H_2S. The largest yield of H_2S obtained in any of the analyses was 0.00856 gm., and the maximum amounts usually ranged from 0.004 to 0.005 gm., averaging about 0.003 gm. at 110° to 115° F. Apparently, the average amount of GSH reacting under these experimental conditions was about 21.4% of the total present in the tissues, and the maximum yield of H_2S was equivalent to 61.5% of the average amount of GSH assayed.

TABLE XV

GLUTATHIONE (GSH) CONTENT AND PH OF PEEL OF FRESHLY PICKED, RIPE EUREKA LEMONS

NUMBER OF SAMPLES ASSAYED	AVERAGE GSH IN PEEL* (FRESH-WEIGHT BASIS)	ACIDITY OF EXTRACT
	mg./100 gm.	*pH*
2	56.8	6.84
4	40.9	6.62
4	35.5	7.17
4	24.1	6.80
4	8.9	6.94
4	16.1	6.82
4	24.4	6.81
Average	29.5	6.83

* Determined by the GUTHRIE (24) method.

Analysis for vitamin C showed no difference between nondusted and sulphur-dusted peel of ripe lemons (80); thus vitamin C probably was not a factor in H_2S production.

If sulphur-dusted fruits are incubated in a jar in which air is free to circulate, some of the glutathione (in reduced form) content of the peel is oxidized, the percentage oxidized depending on the duration and temperature of the incubation period, as shown in table XVI.

The system $RSSR + H_2S \leftrightarrows 2\ RSH + S$ is presumably a reversible reaction. It was shown by SMYTHE (68) that cysteine in the presence of desulphurase reacted with H_2S* to give radioactive cysteine, suggesting the reversibility of the above reaction, but he was not certain whether this completely proved the point of reversibility or whether other reactions were involved.

When sulphur injury occurs to citrus fruit in the field, the conditions are probably neither strictly aerobic nor anaerobic. Injury generally occurs when there is little air circulation, the gases formed in the fruit, after escaping from the cells of the peel, possibly remain in the peel intercellular spaces in high concentration, though they are, nevertheless, free to diffuse into the air through the stomata of the fruit peel.

TABLE XVI

OXIDATION OF GLUTATHIONE (GSH) AS INDICATED BY THE GSH CONTENT OF PEEL OF SULPHUR-DUSTED EUREKA LEMONS AND NAVEL ORANGES INCUBATED FOR VARIOUS LENGTHS OF TIME AT VARIOUS TEMPERATURES (FRESH-WEIGHT BASIS)

KIND OF FRUIT	GSH IN PEEL OF FRUIT NONDUSTED, NONINCUBATED	S-DUSTED FRUIT			GSH OXIDIZED
		INCUBATION		GSH IN PEEL	
	mg./100 gm.	*hours*	° F	*mg./100 gm.*	%
Lemons	61.4	24	105	6.1	90.1
Oranges	61.1	5	140	59.1	3.3

It is calculated that for a Valencia orange 2.5 inches in diameter, with an atmosphere of H_2S 1 mm. in thickness around it, at 86° F (atmospheric pressure, 760 mm. Hg; relative humidity of the air, 50%; peel temperature, 120° F), and with a maximum of 3.4 μg. H_2S produced per fruit per hour, the concentration of gas at the end of one hour would be 294 p.p.m. This seems to indicate that although sulphur could oxidize reduced glutathione, the H_2S produced, if not removed rapidly, might tend to force the equilibrium in the reverse direction. In order to test this possibility, sulphur-dusted and nondusted fruits were sealed in jars, incubated at 140° F for several hours, and the glutathione content of the peel was determined by BINET and WELLER'S (**4**) cadmium lactate method. The results (table XVII) show that there was a substantial increase in the reduced glutathione present, and in view of SMYTHE'S work (**68**), suggest the presence of the enzyme desulphurase. It is possible that where aeration of fruit treated with sulphur is not rapid, a decrease in H_2S formation may take place. This factor should probably be taken into account in the experiments mentioned earlier. There is a rough seasonal correlation between glutathione (reduced) content of the peel of lemons (table XVII) and the H_2S produced by sulphur-dust, as shown in figure 9.

TABLE XVII

GLUTATHIONE (GSH) CONTENT OF PEEL OF NONDUSTED AND SULPHUR-DUSTED EUREKA LEMONS INCUBATED IN TIGHTLY SEALED JARS AT 140° F* (FRESH-WEIGHT BASIS)

SAMPLING DATE	NUMBER OF SAMPLES	GSH IN PEEL OF NON-INCUBATED NONDUSTED FRUIT	GSH IN PEEL OF INCUBATED FRUIT		
			INCUBATION PERIOD	NON-DUSTED	S-DUSTED
		mg./100 gm.	*hours*	*mg./100 gm.*	*mg./100 gm.*
Oct. 4 to 12, 1945	7	24.05	0	.....	.....
Oct. 16 to Nov. 19, 1945	62	16.02	0	.....	.....
Nov. 20 to Dec. 7, 1945	7	43.60	3	66.84	82.04
Dec. 10, 1945 to Jan. 9, 1946	9	40.22	4	72.11	84.93
Jan. 11 to 18, 1946	4	36.68	5	68.12	85.56

* Determined by BINET and WELLER'S (4) cadmium lactate method.

OXIDATION-REDUCTION POTENTIALS

RYKLAN and SCHMIDT (**58**) measured the E.M.F. of solutions of glutathione, having various degrees of oxidation at concentrations of 0.676 mg./ml. The present author has determined, by the use of a glass electrode, that the oxidation-reduction potentials of water extracts of the peel of sulphur-dusted and nondusted lemons incubated for 48 hours in sealed containers at temperatures ranging from 86° to 131° F were lower than extracts from fresh peel (table XVIII). The lower potentials are indicative of in-

TABLE XVIII

AVERAGE OXIDATION-REDUCTION POTENTIALS OF WATER EXTRACTS OF PEEL OF NONDUSTED AND SULPHUR-DUSTED EUREKA LEMONS INCUBATED IN TIGHTLY SEALED JARS FOR 48 HOURS AT VARIOUS TEMPERATURES

TEMPERATURE	NONINCUBATED, NONDUSTED FRUIT	INCUBATED FRUIT	
		NONDUSTED	S-DUSTED
°F	+mv.	+mv.	+mv.
70	26.7	.....	.....
86	.....	23.0	18.8
95	.....	23.7	23.9
104	.....	26.5	24.0
113	.....	18.7	13.0
122	.....	15.2	17.5
131	.....	23.7	24.0
140	.....	32.2	31.2

creased percentages of reduced constituents. At 140° F the extracts of the incubated peels, whether dusted or not, had higher potentials than extracts of fresh peel (table XVIII). Oxidation of some of the reducing constituents of the peel occurred at this temperature, and apparently correlates with the excessive water loss at this high temperature.

Water extracts of the peel of sulphur-dusted fruit incubated at 95° to 104° F had a pH ranging from 4.6 to 4.8, and those of sulphur-dusted fruit incubated at 122° F had a pH of 3.4 to 3.8. On the basis of curves given by RYKLAN and SCHMIDT (**58**), it is estimated that, at the average pH values of the water extracts of the peel of these two groups of fruit, the E.M.F.'s found in this experiment corresponded to 80% and 40% oxidation of glutathione, respectively. This occurrence seems to explain the results of the GSH analyses in table XVII, that H_2S production is low in sulphur-dusted fruit, because when incubation temperatures are in the 95°–104° F range and the pH of the peel tissue fluid is high (4.6 to 4.8),

TABLE XIX

RATE OF WEIGHT LOSSES OF NONDUSTED AND SULPHUR-DUSTED EUREKA LEMONS WHEN INCUBATED FOR 48 HOURS AT VARIOUS TEMPERATURES

TEMPERATURE	MEAN RATE OF WEIGHT LOSS OF FRUIT	
	NONDUSTED	S-DUSTED
°F	mg./gm./hour	mg./gm./hour
75	0.41	.....
86	1.16	1.22
95	1.53	1.75
104	2.36	2.69
113	3.30	3.36
122	4.44	4.64
131	5.63	5.56
140	6.61	6.53
140	2.09*	1.54

* Incubated for three hours only.

more of the GSH is in the oxidized form. When incubation temperatures are high and the pH of the tissue fluid is low, more glutathione is in the reduced form.

Increase in weight loss of fruit

Eureka lemons, when incubated in a constant-temperature oven over a range of temperatures which the fruit attains in the field when in the direct rays of the sun, showed an increasing rate of weight loss as the temperature increased (table XIX). In the temperature range of 86° to 122° F, sulphur-dusted fruit had a higher rate of weight loss than nondusted fruit; above 122°, the nondusted fruit had the higher rate of weight loss.

It has been shown by Wagner (**84**) that the transpiration rate of vegetables is increased by inert dusts, and Duggar and Cooley (**9**) have shown that lime-sulphur sprays increase the transpiration rate of potatoes. Whether or not the effect of sulphur dust on lemons is greater than that of an inert dust has not yet been determined. At this time it seems important to note only that the rate of weight loss is increased by sulphur dust at the critical temperatures at which injury occurs.

The greater proportion of weight loss appears to be due to water vapor, but there are probably also losses due to volatile oils; to gases formed, such as H_2S and SO_2; to a foul-smelling gas which has not been identified, but which passes through a gas mask designed for acid gases; to NH_3; to CO_2 from respiration and perhaps decarboxylation; and to an exudate that appears to be composed of several nonvolatile constituents.

Effect on fruit exudation rate and composition

Citrus fruit confined in closed bottles at temperatures of 105° to 140° F, and either aerated or not aerated, exudes a colorless or yellowish liquid which collects in the bottom of the bottles. Nondusted Washington Navel oranges yielded a colorless exudate at a mean rate per fruit, of 113.9 μg./gm./hour; sulphur-dusted fruit, on the other hand, yielded a yellow exudate at a mean rate of 71.3 μg./gm./hour (table XX).

Both nondusted and sulphur-dusted Eureka lemons yielded a light-yellow exudate, the exudate of the sulphur-dusted fruit usually being the darker of the two. In larger volumes, however, the exuded solutions were deep red by transmitted light. On the average, nondusted fruit yielded approximately 2.69 ml. of exudate per fruit, and sulphur-dusted fruit yielded approximately 2.19 ml. of exudate per fruit.

The exudate from sulphur-dusted lemons contained an average of 0.0350 gm. of soluble solids per milliliter, whereas that from nondusted lemons contained 0.0317 gm./ml. Ash of the exudate from the sulphur-dusted fruit averaged 0.0056 gm./ml., and that from the nondusted fruit averaged 0.0068 gm./ml., with a larger quantity of organic constituents in the exudate from sulphur-dusted fruit (0.0294 gm./ml.) than in the nondusted fruit (0.0249 gm./ml.). Analysis indicated about 0.017 gm. of sugars per ml.; 0.018 gm. of alcohol-precipitable material per ml. (apparently pento-

sans, as they yielded furfural on hydrolysis with phosphoric acid); and 0.0005 gm. of ether extractives per ml. Approximately 0.0032 gm. of sodium (as Na_2SO_4) per ml. was obtained by precipitation with lead acetate and crystallization from sulphuric acid. Organic and inorganic acids were also demonstrated to be present in the exudate.

A qualitative analysis of the exudate ash from both sulphur-dusted and nondusted fruits, incubated at 105° F in closed jars, indicated the presence of iron, calcium, magnesium, sodium, potassium, free ammonium ion, and a trace of aluminum ions. Chlorides, phosphates, sulphates, oxalates, and sulphides were also present in both exudates. It is interesting to note in this connection that bacteria killed by heat are known to lose about half of their salts (**22**). The presence of free ammonium ion is of special significance because it indicates that NH_3 gas may be a product of proteolysis of

TABLE XX

RATE OF EXUDATION BY NONDUSTED AND SULPHUR-DUSTED WASHINGTON NAVEL ORANGES INCUBATED IN TIGHTLY SEALED JARS AT 123.8° F FOR VARIOUS LENGTHS OF TIME

Incubation period	Nondusted fruits				S-dusted fruits			
	Number	Total weight	Exudate		Number	Total weight	Exudate	
			Total weight	Rate of formation			Total weight	Rate of formation
hours		*gm.*	*gm.*	*μg./gm./hour*		*gm.*	*gm.*	*μg./gm./hour*
70.5	8	935.8	7.9	119.7	9	1,209.7	6.1	71.5
79.0	7	1,044.5	12.8	155.1	7	1,050.6	9.0	108.5
100.0	6	864.6	9.6	111.0	6	856.0	6.0	70.1
312.0	6	967.4	20.2	66.9	6	891.8	9.8	35.2
Average	...	.....	.....	113.9	...	.....	.....	71.3

glutamine accompanying the accentuated catabolism brought on by heating and by sulphur treatments.

The presence of the amino acids glutamine and aspargine were found in the exudate from lemons, using the method of GREENHILL and CHIBNALL (**20**). Paper chromatographs indicated possibly seven amino acids, six of which were asparagine, lysine, tryptophane, arginine, histidine and glutamine, but only asparagine and glutamine, and aspartic and glutamic acids were positively identified. The other amino acid was probably stachydrine, which according to NELSON *et al.* 1933, is the predominating base in orange juice. CURTIS (**7**) found several of the ions noted above, and also glutamine, in the deposits left by exudates from lawn grass. Nitrogen determinations (micro-Kjeldahl) on the exudate from nondusted, incubated lemons showed 0.404 mg. N/ml. and 0.481 mg. N/ml. for exudate from the sulphur-dusted, incubated fruit. The Aeree-Rosenheim, and the Liebermann xanthoproteic, reactions were positive for tryptophane in exudates from both sulphur-dusted and nondusted lemons, but the bromine water test was negative. Tests for nitrates by four different methods gave positive results

for the exudate of both sulphur-dusted and nondusted fruit by the first method (L. de Nando), indecisive results for both exudates by the second method (ring test), indecisive results for the exudate of sulphur-dusted and negative results for the nondusted by the third method (spot test), and negative results of the exudates of both sulphur-dusted and nondusted fruit by the fourth (varnished-slide test). If, therefore, nitrates are present in the exudate, they are present in limited quantities. Tests for nitrites were negative in the exudates from both sulphur-dusted and nondusted lemons.

The sugars in the exudate were tentatively identified by means of their osazones as fructose, glucose, arabinose, galactose, and possibly maltose. Since the known sugars present in juice are sucrose, glucose and levulose, the sugars in the exudate appear to be those derived from the acid hydrolysis of sucrose and pectin. A large part of the red-brown color present in the original exudate remained with the sugars. Spectrographic analysis of the crude-colored exudate gave a gradually rising curve with 100% absorption from 300 to 375 mμ and 94% transmission at 700 mμ for the sulphur-dusted fruit. The exudate from nondusted fruit gave 100% absorption from 300 to 400 mμ and 92% transmission at 700 mμ. In general, the purified sugar solutions gave a curve somewhat similar in shape to that of the transmission spectrum of the crude exudate. The slight differences in the details of the shapes of the curves, however, together with the 145-mμ difference in absorption maxima (100% absorption from 300 to 520 mμ and 56% transmission at 700 mμ), indicate the presence of other chromaphore groups. While catalase in dilute solution is yellow (**69**), and while the presence of catalase was demonstrated in the exudate, as indicated later, its absorption bands at 627, 536, and 502 mμ do not correspond with absorption spectra of partially purified exudate. Hydrogen sulphide catalase has absorption bands at 640.5, 587, and 548 mμ, which likewise do not correspond with the spectra of the exudate. The principal color constituent in the acid exudate appears to be due to humus substances. These are generally formed by the action of heat and mineral acids on sugars and are not well understood, (**5**).

GORTNER *et al.*, working on a related problem for a number of years, found that humin (melanin) nitrogen is formed when proteins are hydrolyzed by strong acids, and they determined that condensation of tryptophane with an aldehyde was responsible. Numerous papers have appeared since, which deal with the Maillard reaction and the browning of proteins. The reaction is known to occur in citrus juice concentrates and powders.[2]

[2] In a private communication H. S. Alcott observed: "The absorption spectra shown in your curves are very similar to those obtained by us with bovine serum albumin that has been allowed to turn brown in contact with aldehydes, in that they are without character in the visible region and show a peak at 275–280 mμ. The 'shelf' on your curves from 310 to 330 mμ resembles one that we observe when acetaldehyde is used for the reaction. Our 'shelf' is from 300–325 mμ. When glucose was used no such discontinuity was observed."

Other yellow constituents, however, are present in the exudate. Flavones give the citric-boric acid reaction in acetone and shift the maximum absorption to 325 mμ or lower. Flavanones give a slightly blue-green color with $FeCl_3$. In addition the exudate clouds on standing as do citrus juices. The thoroughly washed insoluble material is slightly soluble in 95% ethanol. The residue on evaporation of the ethanol gives a yellow color with NaOH. The bulk of the material dissolves readily in NaOH giving a yellow solution with maximum absorptions at 325 and 350 mμ for sulphur-dusted and nondusted fruit respectively. A mixture of ascorbic acid, H_2SO_4 and l-tryptophane after incubation 15 hours at 45° C gives a spectrum closely paralleling the curve for the insoluble material (dissolved in NaOH) formed in the exudate of sulphur-dusted lemons. A similar mixture in which dl-trypthophane replaces the l-tryptophane closely parallels the spectral transmission curve of the insoluble material (dissolved in NaOH) in the exudate from nondusted fruit. Pure hesperidin dissolved in NaOH has a maximum absorption at 400 mμ and more nearly parallels the curves determined for the pure exudate.

The exudate from sulphur-dusted lemons had a pH of 3.40, and that from nondusted fruit had a pH of 3.48, at 15° C. The exudate from the former required 0.219 m.e. of NaOH, and that from the latter required 0.191 m.e., to neutralize 1 ml. to the phenolphthalein end point. The exudate from sulphur-dusted Washington Navel oranges had a pH of 4.05 at 15° C, and required 0.0144 m.e. of NaOH to neutralize 1 ml., while that from the nondusted fruit had a pH of 4.10 and required 0.0108 m.e. of NaOH.

It appears from the results of the above analyses that lemons, which are quite susceptible to sulphur injury, release more acid and a greater concentration of acid than Washington Navel oranges, which are not so susceptible. Likewise, lemons, when sulphur-dusted and heated, release an exudate much more acid, in comparison with that of nondusted fruit, than was found with Washington Navel oranges subjected to similar treatments.

A preliminary identification of the acids present in the lead acetate precipitate from 1 ml. of the exudate indicated approximately 438 μg. of phosphoric acid and 4.82 μg. of malic acid. Oxalic acid, sulphuric acid, and two or more unidentified acids (possibly citric, glutamic, aspartic and pyruvic acids) accounted for an equivalent of 0.774 m.e. of NaOH.

The analyses of the exudates show that lemon fruits are especially susceptible to heat, which causes them to lose water, minerals, salts, sugars, and amino and other organic and inorganic acids. Sulphur dust reduces the volume of the exudate and minerals but increases the total soluble solids exuded. Qualitatively, the exudates from treated and control fruit appear to be the same. It was hoped that some qualitative difference might be found thus furnishing a lead to the effect of sulphur. In this rather thorough analysis only quantitative differences have been found. A complete quantitative characterization of the exudates will be given in a subsequent paper.

EFFECT ON SOLUBILITY OF MINERAL ELEMENTS IN PEEL TISSUE FLUID

Treatment of citrus fruits with elemental sulphur is followed by production of H_2S gas. This gas is released in quantity. Vacuum is not required to remove it from the fruit. It appears then that H_2S must emanate from the cell solution which we can reasonably assume to be saturated with respect to this gas. In view of the acidity of the cell solution after dusting of the fruit with sulphur, precipitation of certain metals in the cell solution would be expected. In fact, MANN and LUTWAK-MANN (**42**) state: "It is perfectly reasonable to assume that the instantaneous and reversible inhibition of an enzyme by metal-binding substances such as cyanide, azide, or hydrogen sulphide usually indicates that the enzyme contains a metal." RODENACKER (**57**) suggested that catalytically active Fe in blood is converted to FeS by H_2S.

In order to determine what metals in citrus fruit are precipitated by sulphur dusting, the following experiment was made. Sulphur-dusted and nondusted Eureka lemons were heated in sealed bottles at 105° F for 72 hours, then peeled, and the peelings squeezed in HCl-washed linen, the tissue fluids being received in unused HCl-washed beakers. Great pains were taken to prevent the minutest contamination of this fluid from any external source during manipulations. After ashing in silica dishes, without filtering, the ash was spectrographed. The results are shown in table XXI.

With six of the elements, there was no difference between percentages in the ash of peel tissue fluids of nondusted and sulphur-dusted fruit. Differences were significant with chromium, nickel, and strontium, which were rendered more soluble in the sulphur-dusted fruit, but these three metals are of no physiological interest. There were small differences in the percentages of silver, boron, copper, manganese, and zinc, which were rendered slightly less soluble, and in molybdenum, which was rendered slightly more soluble, in the sulphur-dusted fruit. These last-named metals, with the exception of silver, are, of course, of considerable physiological interest because of their catalytic effects. Boron is an essential minor element (**26**) which plays a role in determining the solubility of calcium (**61**), and may play a role in the formation of pectin compounds (**26**). REED (**54**) states that in boron-deficient plants the hydrogenase system fails, and the phosphate distribution is abnormal. Copper is known to be a constituent of potato oxidase (**50**) and other enzymes such as ascorbic acid oxidase and tyrosinase (**69**). Both copper and zinc are known to catalyze the oxidation of RSH, their activities being inhibited by H_2S (**44**). Manganese is an essential minor element (**41**) having a role in the enzyme system responsible for the reduction of nitrates (**6**), and molybdenum has recently been shown to be an essential minor element in citrus (**82**). It therefore appears that sulphur may exert an action either directly or indirectly which disturbs the role of minor elements in certain of the fruit enzyme systems.

TABLE XXI

SPECTOGRAPHIC ANALYSIS (IN PERCENTAGE OF ASH) OF HAND-EXPRESSED TISSUE FLUID OF THE PEEL OF EUREKA LEMONS, NONDUSTED AND SULPHUR-DUSTED AND INCUBATED IN TIGHTLY SEALED JARS FOR 72 HOURS AT 105° F

FRUIT TREATMENT	Ag	Al	B	Ba	Cr	Cu	Fe	Mn	Mo	Ni	Pb	Sn	Sr	Ti	Zn
	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
Incubated, Nondusted	0.0006	0.07	0.05	0.012	0.005	0.015	0.1	0.05	0.001	0.0005	0.003	0.006	0.2	0.005	0.05
Incubated S-dusted	0.0005	0.07	0.04	0.012	0.015	0.012	0.1	0.03	0.0015	0.001	0.003	0.006	0.4	0.005	0.04

Effect on enzymes

Zeile and Hellstrom (**89**) found that high temperatures, certain acids, and small amounts of H_2S inhibited catalase activity. Oppenheimer and Stern (**50**) state that H_2S inhibits peroxidase, catalase, and indophenol oxidase (cytochrome oxidase). Day and Franklin (**8**) found that carbonic anhydrase, which contains zinc, was sharply inhibited by H_2S, and H_2S is known to inhibit dipeptidase and dopa oxidase. Hussein, Mrak, and Cruess (**28**) found that SO_2 inhibited oxidase activity in raisins.

Freezing microtome and freehand sections were made of fresh lemon peel and of peel of nondusted or sulphur-dusted fruit that had been incubated at 105° F for 24 hours in closed containers. Tests for enzymes were made according to standard microchemical methods; the results are shown in table XXII. Catalase in these sections appeared to be inactivated by the treatment with sulphur dust. However, determination, with a Warburg apparatus, of catalase in the exudate from sulphur-dusted and nondusted lemons showed that the catalase activity of exudate of sulphur-dusted fruit was only slightly less than in the nondusted. It was shown by Bernheim,

TABLE XXII

Activity of oxidizing enzymes in peel of nondusted and sulphur-dusted Eureka lemons incubated for 24 hours at 105° F

Oxidizing enzyme	Fruit nonincubated, nondusted	Incubated fruit	
		Nondusted	S-dusted
Indophenol oxidase	−	−	−
Peroxidase	+	+	+
Catalase	+	+	−

Bernheim and Gillaspie (**3**) that d-amino acid oxidase free of catalase oxidizes d-methionine with the liberation of H_2O_2. Inactivation of catalase by H_2S in the present experiments suggests that H_2O_2 might be liberated and result in the oxidation of ^-SH groups.

A preliminary attempt was made to locate the points at which enzymes present in lemon peel were effective in producing gaseous products and SO_4 from sulphur and its compounds. Enzymes were inactivated by heating Eureka lemons for 5½ hours in an oven at 100° C. At the end of this period the center of the fruit had attained a temperature of 69° C, sufficient to inactivate most of the enzymes. Fruit which had been preheated, and nonpreheated controls, were then sulphur-dusted and incubated at 105° F, or incubated in H_2S or SO_2 gas at a pressure of 1 mm. of Hg. The preheated fruit produced less H_2S and SO_2, and a lower percentage of sulphur as SO_4, than the nonheated controls. Preheated fruit gassed with H_2S produced a lower percentage of sulphur as SO_4 than the nonpreheated fruit, while the preheated fruit gassed with SO_2 gave a slightly smaller amount of H_2S than the control. However, the percentage of sulphur as SO_4 was much lower in the preheated fruit than in the control (table XXIII).

These experiments suggest that the formation of H_2S from elemental sulphur is enzymatic, that the oxidation of H_2S to SO_4 is enzymatic, and that the oxidation of SO_2 to SO_4 is enzymatic.

TABLE XXIII

INACTIVATION OF ENZYMES IN EUREKA LEMONS BY PREHEATING FRUIT FOR 5½ HOURS AT 100° C, AS INDICATED BY SULPHUR PRODUCTS FORMED ON SUBSEQUENT TREATMENT WITH SULPHUR DUST OR WITH H_2S OR SO_2 GAS (1-CM. PRESSURE) AND INCUBATION AT 105° F FOR VARIOUS LENGTHS OF TIME

PRELIMINARY TREATMENT OF FRUIT SAMPLES*	GASEOUS PRODUCTS FORMED			ACIDITY OF PEEL EXTRACT	S (DETERMINED AS SO_4) IN DRY PEEL
	TOTAL H_2S	TOTAL SO_2	TOTAL NH_3		
	mg.	*mg.*	*mg.*	*pH*	%
Fruit sulphur-dusted; incubated 67 hours					
Not preheated	42.59	23.74	.....	3.70	0.1379
Preheated	0.23	0.09	49.05	3.00	0.0989
Fruit incubated 18 hours in H_2S gas					
Not preheated	.....	1.43	.....	4.50	0.1696
Preheated	.....	.....	.....	3.45	0.1062
Fruit incubated 17 hours in SO_2 gas					
Not preheated	19.55	.....	.....	3.60	0.2353
Preheated	15.81	.....	.....	3.00	0.1379

* Each sample consisted of 28 lemon fruits.

SULPHUR VAPORIZED VERSUS SULPHUR RECOVERED

The relative amount of sulphur dust vaporized after application to a lemon fruit, as compared with the total sulphur recovered in the experiments, is indicative of the relative status of the problem. Calculations show that 7.5 mg. of this sulphur per fruit per 72 hours would sublime and be available for reaction with the lemon tissue. Analyses indicate that 0.661 mg. of sulphur is recoverable from the peel as SO_4, that 0.0084 mg. is recoverable as SO_2, and that 0.268 mg. is recoverable as H_2S. This accounts for 0.9374 mg. of sulphur and leaves 6.5626 mg. unaccounted for. No doubt some of the sulphur, perhaps half of it, can be obtained as sulphur vapor; some of it probably is in organic combination in certain proteins and amino acids. Possibly some of it is combined in the volatile constituent of the peel which gives the foul-smelling gas that is not absorbed; while another portion of it is to be found in the exudate.

The sulphur recoverable from the peel as sulphate, if produced from the sulphur applied to the peel as sulphur-dust, and if not neutralized, or hampered by the buffer system of the peel, is sufficient to lower the pH to 3.19. When the peel is incubated for 48 hours at 113° F, the water vapor losses are sufficient to lower the pH to 2.97, on the basis of activity coefficients given by LEWIS and RANDALL (**35**).

TEMPERATURE AND RADIATION IN SULPHUR INJURY

The rate of change of temperature of lemons and oranges is of considerable importance in laboratory experiments on the physiology of sulphur injury, and it probably is of equal importance in sulphur injury to fruit in the orange or lemon grove. The volume of a lemon nearing maturity is about 100 ml., its specific gravity varies from 0.91 to 0.95 (**76**), and its specific heat is 0.700 (**33**). The peel has a compact outer layer (flavedo) which probably conducts heat rather readily, but the structure of the inner peel (albedo), interspersed as it is with large intercellular spaces (**79**), probably forms a poor medium for heat conduction.

Temperatures of detached oranges and lemons in the laboratory, and of fruit on the trees in commercial groves, were studied by means of copper-constantan thermocouples and a potentiometer of high sensitivity.

In figure 12 is shown the increase in the temperature of the albedo of Eureka lemons with time, when enclosed in a constant-temperature chamber at 90° F (32.22° C). In contrast to this curve, the curves shown in figure 13 indicate a very rapid rise in the temperature of the flavedo (ther-

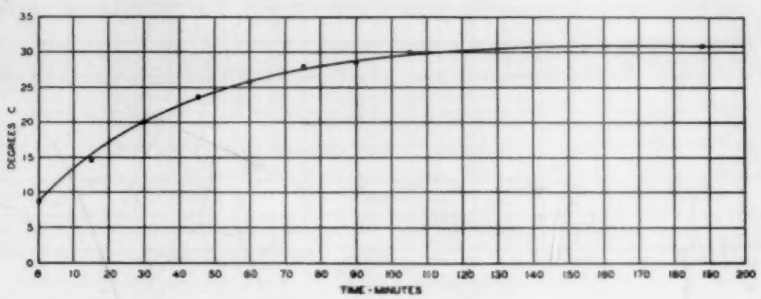


FIG. 12. Average temperature changes per minute in the albedo of Eureka lemons held in a constant-temperature chamber at 90° F (32.22° C).

mocouples about 500 μ from the surface) a few seconds after immersion in a water bath maintained at 118° F. As indicated by these curves, the initial rise in the H_2S and SO_2 curves (figure 11) correlates with the increase in temperature of the peel of the fruit.

Temperature measurements of the sides of lemon fruits exposed to the direct rays of the sun have given values as high as 127.7° F when the air temperature was 104.7° F (**80**). Inasmuch as air temperatures in the Riverside area not uncommonly exceed 110° F, and sometimes 115° F, we can reasonably expect fruit-peel temperatures in this area on the order of 140° F (**80**). These measurements, and the general observations of citrus growers that sulphur injury does not occur until air temperature reaches 100° F or above, were the basis for the incubation temperatures used in the experiments reported.

In an extensive field experiment it was shown that 0.15% of the lemons which had no treatment "burned" from the rays of the sun alone. Of

those which had been dusted with elemental sulphur dust, 9.88% were burned (**80**). One interpretation of these data is that sulphur lowered the critical temperature at which the fruit was susceptible to heat injury. Such an interpretation is fortified by the fact that, under ultraviolet filters, 6.43% of the nondusted fruit was injured, while 10.54% of the sulphur-dusted fruit was burned (**80**). Other parts of the same experiment, in

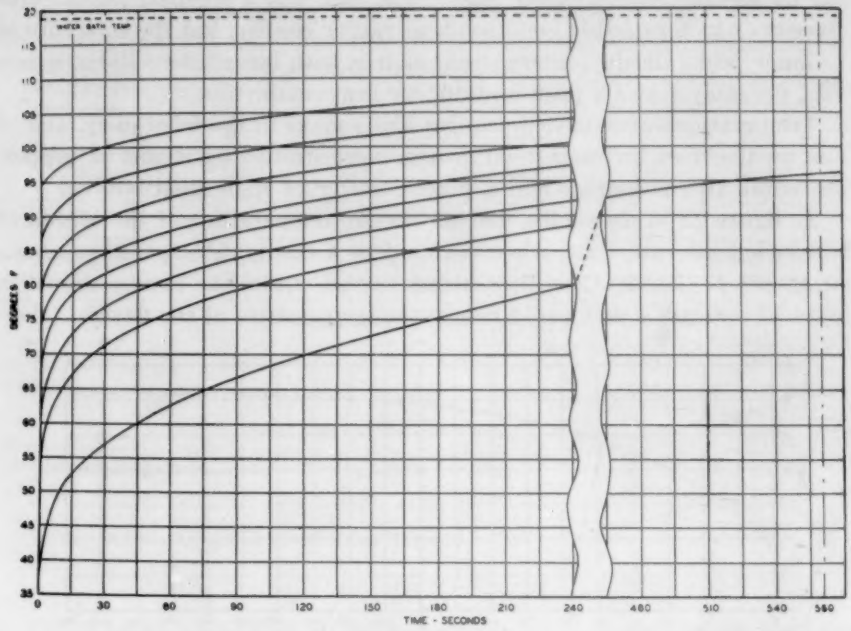


FIG. 13. Average temperature changes per second in the flavedo of Eureka lemons immersed in a water bath maintained at 118° F (thermocouples about 500 μ from surface of fruit). Fruits were in equilibrium with various temperatures before immersion.

which trees were covered with cheesecloth tents, indicated that this interpretation was tenable. Two days prior to a hot spell the sun radiation was 671 and 649 gm. cal./cm.^{2}/day, but the air temperatures were 78° and 87° F, respectively, and no burning of the lemon fruit occurred. On the two following days the sun radiation dropped to 629 and 634 gm. cal./cm.^{2}/day, and the air temperatures were 104° and 108° F, respectively. Sunburn and sulphur burn were in increasing evidence during and after these two days.

Taking other meteorological factors into consideration, it was evident that sun-radiation intensity was not the causal factor *per se*. Its effect was conditional upon air temperature, and injurious action was induced by affecting fruit temperature. It appears from these data that, inasmuch as the relative humidity was 4% to 6%, the ratio of incoming radiation to outgoing radiation (largely contact conduction)—that is, the rate of heat absorption relative to the rate of heat dissipation—determined whether or

not injury would occur. In these field experiments warm air reduced the heat loss from the fruit so that injurious temperatures were attained in the fruit peel.

The effect of interference with reradiation from the fruit of the energy derived from incoming sun radiation was studied by interposing light-transparent (yellow cellulose acetate sheeting) alternately with light-opaque (metal) radiation shields between a lemon fruit and the direct rays of the full summer sun, and recording the peel temperatures thermoelectrically. The results of such an experiment are shown in figure 14. The

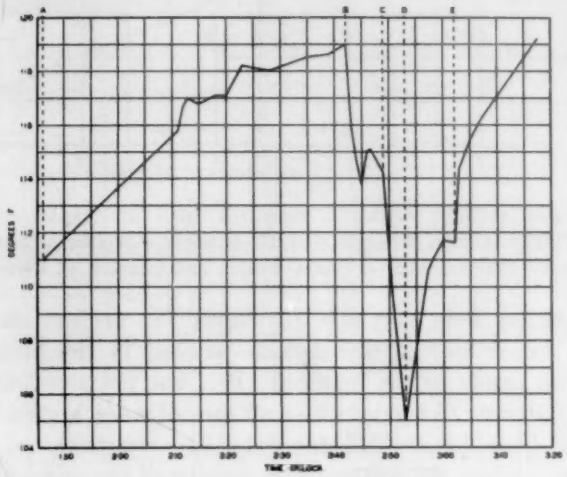


FIG. 14. The effects of light-transparent and light-opaque radiation shields on the temperature of the albedo of a Eureka lemon fruit in full summer sun. Transparent shield (yellow cellulose acetate sheeting) placed between sun and lemon at A; removed at B. Metal shield interposed between sun and lemon at C; removed at D. Transparent shield interposed again at E.

mechanism of the temperature increase in the fruit peel is, briefly, that the acetate shield transmits less than 5% of sunlight having a wave length of 500 $\mu\mu$, but transmits 75% to 95% of the sunlight having wave lengths of 550 to 700 $\mu\mu$. The energy of these wave lengths is converted into terrestrial radiation by the fruit, according to Wien's displacement law ($\lambda_m T = b$). The wave length in cm. (λ_m) is a function of the absolute temperature (T) of the body, and the constant b = 0.288 cm. deg. Calculations for fruit temperatures of 70° and 140° F indicate that the fruit would reradiate in a range of wave lengths of 9.8 μ to 8.7 μ, which lie in the infrared portion of the spectrum. Radiation of wave lengths of 7.0 μ to 11.0 μ is absorbed 100% by the shield, which radiates back to the lemon fruit and thus raises its temperature. Calculation also indicates that the trapping of water vapor under the filter is likewise a factor. The mathematics for the behavior of such a shield has recently been published by LAWSON and FANO (**34**).

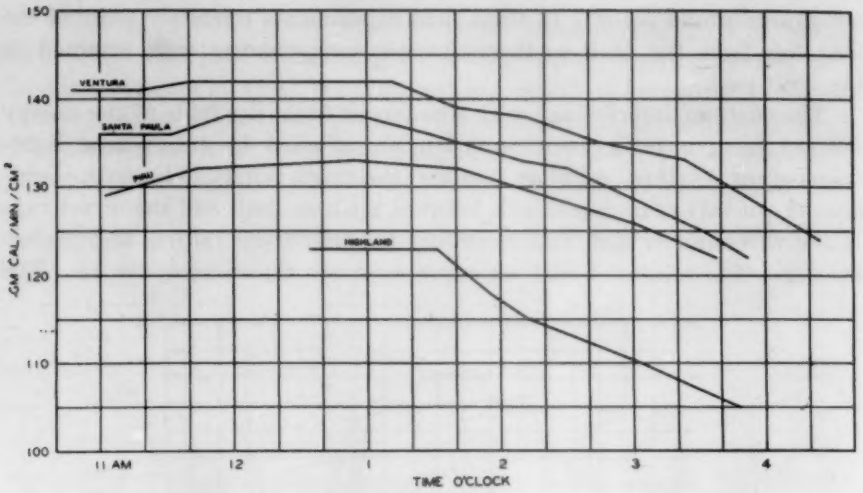


FIG. 15. Total sunlight intensity, measured with an Eppley thermopile, for warmest days (1941) in three different climatic districts in southern California: coastal district, at Ventura; intermediate, at Santa Paula; and interior, at Piru and Highland.

Suffice it to say here that this is roughly the mechanism of the heat trap. Water vapor and carbon dioxide present in the atmosphere also have absorption bands in the infrared (**10**), but the absorption of water vapor in this portion (9.8 μ to 8.7 μ) of the infrared region is relatively slight, ranging from 3% to 61% for water-vapor densities of 0.21 to 4.15 gm./cm.^{2} (**10**). When the water-vapor density of the atmosphere is high, the water vapor probably also acts as a heat trap.

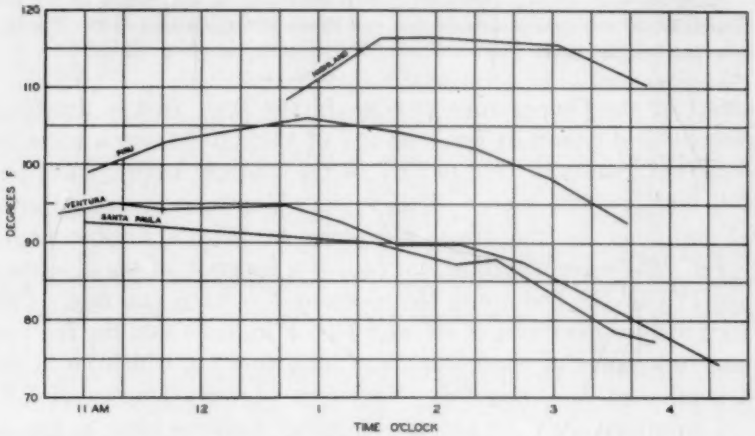


FIG. 16. Peel temperatures of Eureka lemons in full sunlight, measured with copper-constantan thermocouples placed in the side of the fruit in the direct sun, on the warmest days (1941) in three different climatic districts in southern California: coastal district, at Ventura; intermediate, at Santa Paula; and interior, at Piru and Highland.

The action of high water-vapor density may partly explain why sulphur damage has been generally observed to be more severe in the coastal than in the interior areas of southern California. Of course the effect of xerophytic conditions existing in interior districts, in hardening plant tissues, must also be recognized. Water-vapor density, however, may also partly explain why leaf damage is severe in humid regions but seldom occurs in the dry interior areas of the state, and why, in direct sunlight, fruit-peel temperatures are higher in the interior regions. Sunlight intensities in three different climatic districts in southern California are shown in figure 15. The highest intensity was at Ventura, in the coastal district; next

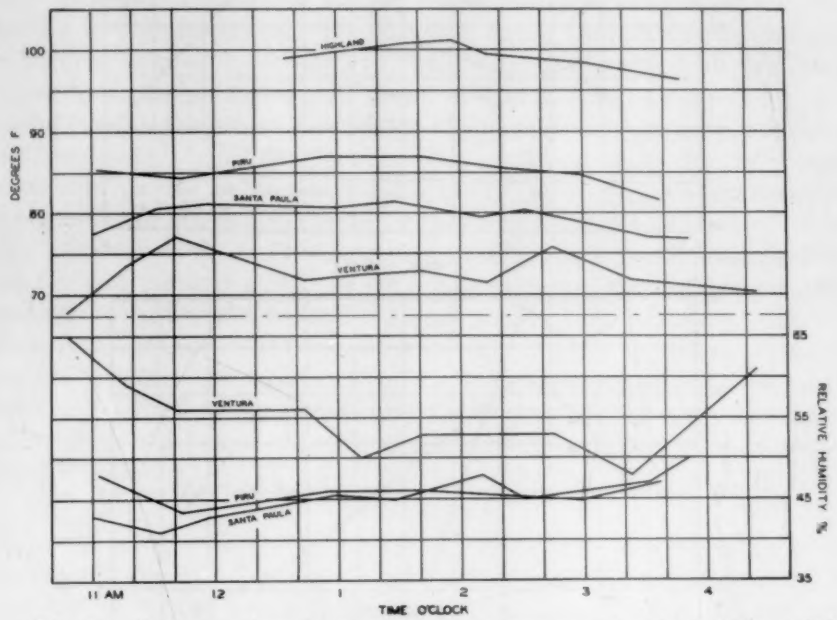
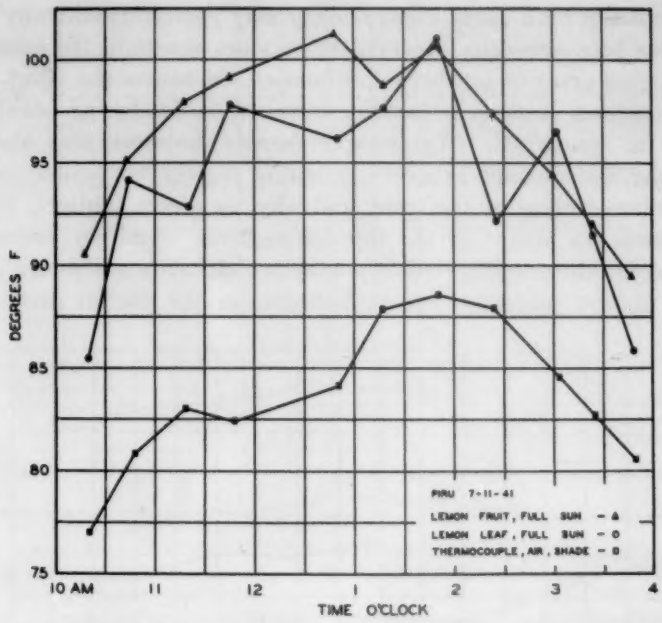
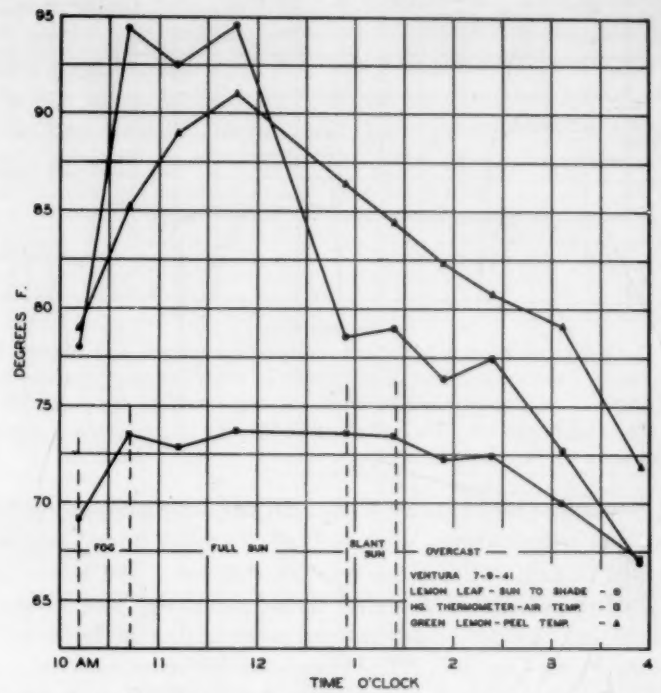


FIG. 17. Air temperatures in the shade (upper four curves) and relative humidities (lower three curves) for the warmest days (1941) in three different climatic districts in southern California: coastal district, at Ventura; intermediate, at Santa Paula; and interior, at Piru and Highland. The relative humidity at Highland (not shown) varied from 11% at noon to 14% at 4 P.M.

highest at Santa Paula, 16 miles inland, in the intermediate district; and the next highest temperatures were at Piru, 35 miles inland, and at Highland, 70 miles inland, both in the interior district. The lemon-peel temperatures during periods of observed light intensities were in the inverse order, except that the peel temperatures were higher in fruit at Ventura than at Santa Paula (fig. 16). The respective air temperatures of all locations are in inverse order to the values for sunlight intensity (fig. 17, upper curves). The higher air temperatures (as well as greater infrared intensity from the sun) in the interior region interfere with the dissipation of heat from the fruit and appear to account for the higher fruit tem-





FIGS. 18, 19. Air temperatures (measured in the shade) and temperatures of a lemon leaf and of a green lemon fruit (peel) in two different districts in southern California. Figure 18 shows results of measurements in full sun in the dry interior district of Piru; figure 19 shows results of measurements on a day of variable fog and sunshine in the humid coastal district of Ventura.

peratures, but they do not account for a higher peel temperature at Ventura, as compared with that at Santa Paula. The relative humidities shown by the lower curves in figure 17 indicate why the lemon-peel temperatures were higher at Ventura than at Santa Paula despite the cooler air temperatures at the former location. The water-vapor density was greater at Ventura and acted as a radiation shield, as explained earlier. The relative humidity at Highland varied from 11% at 12 M. to 14% at 4 P.M. and is not shown in figure 17.

Numerous examples may be cited to show how vapor density affects terrestrial temperatures, but the following may serve to explain the conditions which are responsible for the severe leaf injury following sulphur dusting in coastal regions. The curves in figure 18 show that in full sun in the dry interior district of Piru temperatures of lemon-fruit peel were higher than those of leaves. As shown in figure 19, there was morning fog in the coastal district at Ventura on the day of the measurements, with correspondingly high relative humidity. (Water-vapor density varied from 6.059 gr./ft.^{3} at 10 A.M. to 5.131 gr./ft.^{3} at 4 P.M.) When temperature measurements were begun in the morning, the fruit-peel temperatures were a little above the leaf temperatures. As the sun began to break through the fog and the leaf and fruit came into full sun, the leaf temperature rose more rapidly, and reached a higher temperature maximum, than the fruit peel. Although these conditions existed only 2½ hours, the leaf reached a higher temperature than the fruit, owing largely to the small mass of the leaf as compared with the fruit. Higher air temperature might bring the leaf to the critical temperature at which it could be injured by sulphur.

Discussion

LEWIS and RANDALL (**35**) state that "There is no element which in its compounds and its elementary forms presents so diversified a series of problems in applied thermodynamics as sulphur." Sulphur likewise presents a diversity of problems in the normal or abnormal metabolism of this element in plants, as well as in living organisms in general. The variability of citrus fruit introduces other problems.

Lemons are very susceptible to injury by sulphur sprays and dusts, but they are extremely variable in respect to the degree of injury and in various localities and to individual fruits, trees, and varieties. Lemon trees produce flowers and fruit the year around. Rates of fruit growth and maturation vary widely and require either the tagging of the flowers or the use of unwieldy, large samples in order to obtain physiologically comparable fruit. In either case, samples, although available the year around, are not similar physiologically or chemically throughout the year.

The variability in the pH of the peel tissue fluid of lemons may be one of the factors affecting the variability in the evolution of H_2S and SO_2 gases. The present determinations show that the peel extract (1:4 dilution) varies from pH 5.60 for small green fruit (2.4 cm. in diameter) to

pH 5.00 for the "silver-stage" and yellow fruit (4.4–5.7 cm. in diameter); thus the maturity of citrus fruits is definitely reflected in the pH of the peel extract, although differences between the pH of the fruit in the silver and yellow stages are not significant. (SLUITER (**65**) has shown that pH has a pronounced effect on the production of H_2S by animal tissues.) Seasonal variation of the glutathione content (in reduced form) of the fruit peel contributed also to the variable production of H_2S.

All these factors have contributed to the variability of the experimental results. Variability has been evaluated by statistical reduction of the data where necessary. In a few cases data of analytical precision have been obtained. Many of the laboratory studies have been carried out under anaerobic or partially anaerobic conditions, but additional experiments are necessary under aerobic conditions in order to further elucidate the physiology of sulphur injury.

Many of these experiments have involved drastic treatments, with the object of obtaining data upon which to base more refined experiments employing less drastic treatments. While the results indicate that the respiratory system of the fruit is involved in sulphur injury, no respiration determinations have been made because it is possible that several gaseous products not yet identified might lead to erroneous observations and interpretations. The reactions of oxygen and sulphur with other substances, and the resulting products (H_2O, H_2S, etc.), are similar in many respects and thereby introduce many complications. In measuring the effect of various sulphur preparations on the respiration of apple leaves, HYRE (**29**) noted no difficulties from sulphur gases at temperatures of 70°, 85°, and 100° F. He reported that sulphur fungicides increased respiration, but the differences were not significant as compared with the untreated controls. His observations indicated that about 40% more leaf injury occurred at 100° F than at the lower temperatures. For him, working in such a low temperature range, gases such as H_2S, SO_2 and NH_3 may not have been troublesome, but the lack of significant differences in his results may have been due to the presence of unknown gaseous products, and, possibly, to the confounding of decarboxylation CO_2 with respiratory CO_2.

It has been assumed that the physiology of sulphur injury in higher plants might be somewhat similar to the physiology of its toxic effects to fungi. MCCALLAN and WILCOXON (**40**) have presented a thorough review of the production of H_2S by living tissues and have shown that the leaves of a number of species of plants produced H_2S when treated with sulphur, and that the spores of a number of fungi also produced H_2S. They found that the optimum temperature for H_2S production was 95° F, and that the reaction was inhibited at 140° F; they therefore concluded that the reaction was enzymatic. The results of the present experiments indicate that in citrus fruits the optimum temperature may be above 120° F, and that temperatures of 212° F bring about at least partial inactivation. Partial inactivation may be due to the fact that, though the outer tissues of the

fruit were inactivated prior to dusting with sulphur, the inner tissues may not have been. Guthrie, on the other hand, in working with potato and gladiola juice, found that boiling before reacting with sulphur increased the production of H_2S. He attributed this difference in unboiled and boiled juice to the action of oxidase (**23**). However, in the present experiments, lemons which were inactivated by placing them in an oven for 18 hours at 212° F, after gassing them with H_2S, had a pH of 3.45 in the peel extract and an SO_4 content of 0.1011% sulphur/gm. dry weight of peel, whereas noninactivated fruit, after gassing with H_2S, had a pH of 4.5 and 0.1196% sulphur/gm. dry weight of peel. That the conversion of H_2S to SO_4 is enzymatic seems probable.

WILLIAMS and YOUNG (**87**) showed that water filtrates from flowers of sulphur and from ground sulphur inhibited the germination of spores more than sulphuric acid of the same normality. The extra activity was attributed to polythionic acids, which along with sulphuric acid have been shown to occur in powdered sulphur. WILCOXON and MCCALLAN (**85**), working with conidia of several species of fungi and with uredospores of *Uromyces* (**77**) showed that the toxicity of the two acids is the same, and that the effects are due to differences in pH. Considerable work has also been done by English workers, and the work on the fungicidal action of sulphur has been well reviewed by FREAR (**14**). Recently, PARKER-RHODES (**51**) showed that spores could absorb only hydrodisulphide, dithionate, pyrosulphite, or thiosulphite ions, and he suggests that sulphur exerts its action as pyrosulphoxylate.

It is improbable that mineral acids dissolved in water readily penetrate the rind of uninjured citrus fruit, as uninjured fruit rind is nearly impervious to water (**76, 79**). However, since H_2S, SO_2 and NH_3 gases evidently are formed in fruit dusted with sulphur and incubated, the whole problem appears in a new light, as these are the products of dismutation of certain thiols (**67**). It is stated by HALL *et al.* (**25**) that H_2S and SO_2 gases yield a more active form of sulphur in reactions with rubber. According to FREUNDLICH (**15**) these two gases in water form pentathionic acid. Although work on the injurious effects of H_2S and SO_2 gases on higher plants has been extensive, no reports on the toxicity of combinations of the two gases appear to be available.

From the experiments in the present work, it is impossible to attribute injury to any of the products formed by the reaction of citrus fruit tissue and elemental sulphur. It has been shown that elemental sulphur dusted on the surface of lemons produces H_2S, SO_2, and H_2SO_4, that H_2S produces SO_2 and H_2SO_4, and that SO_2 produces H_2SO_4. This suggests that the end product of the metabolism of S, H_2S, or SO_2 is H_2SO_4.

The oxidation of elemental sulphur, H_2S and SO_2 to SO_4 may play an important role in sulphur injury because of the heat liberated in the process. In the field experiment, fruit peel temperatures of fruit in the direct sun were 10° to 15° F above air temperature. No untreated fruit burned

under cheesecloth, but nondusted, noncovered fruit burned, as did sulphur-dusted fruit even though covered by cheesecloth. The peel temperatures of fruit under cheesecloth ranged 1° to 2° F above air temperature. On this basis, treatment with sulphur dust appears to lower the critical temperature of fruit injury 8° to 13° F. Keil's calculations (**32**) showed considerable heat liberation in the oxidation of sulphur by bacteria. Similar calculations based on the increased SO_4 found in the peel of sulphur-dusted lemons, indicates approximately 3.98 calories are liberated. While the temperature of the peel of a 100-gm. fruit would only be raised 0.32° F, a hundred times the volume of tissue shown to be injured in figure 4, would be raised 19.3° F during SO_4 formation.

Because of the poor buffer capacity of the peel of lemons, the pH change in the tissue fluid is large. In fact, the pH often demonstrably passes the isoelectric point of citrus protein. In cases where it doesn't, higher concentration of cations are thought to be present. SINCLAIR (**62**) found that the isoelectric point of the protein of the edible portion of the lemon was at pH 5.6 to 5.7, and that the protein decomposed on heating. This relatively high pH, and the fact that lemon protein decomposes on heating, may account in a large measure for the susceptibility of lemons to sulphur injury. For the edible portion of oranges, SMITH (**66**) found that the isoelectric point of the protein was at pH 4.7. The fact that this protein is not coagulated by heat at neutral, acid, or alkaline reactions may account in part for the resistance of oranges to sulphur injury. These characteristics were confirmed by SINCLAIR *et al.* (**63**). The present studies have shown that several of the reactions between the constituents of citrus fruits and sulphur are accelerated by increasing temperatures, and that two of the reactions (conversion of S to H_2S, and of H_2S to SO_4) are partly inhibited by high temperatures.

In addition to possible specific reactions resulting in injury by sulphur dust, such as precipitation of proteins by acids, protein deamination or decomposition, and amino acid dismutation as indicated by the detection of ammonium ion in the fruit exudates, there is also the possibility that precipitation of such minor elements as copper, zinc, and manganese may inactivate the enzyme systems with which they are correlated, as shown by LOHMANN (**37**) for the inhibition of glyoxalase by M/1,000 Cu, Ag and Hg; and that the increased solubility of metals such as nickel, chromium, and strontium may block the S–H groups and result in an insoluble mercaptide, as shown for Co and Ni salts by GRIFFITH *et al.* (**21**), in a manner similar to that of mercury, as reported by FILDES (**11**).

That the production of H_2S gas by sulphur-treated organic tissue is derived from the reaction of sulphur with glutathione and other S-H-containing organic compounds is well established. The mechanism for the conversion of thiosulphate and other sulphur-containing compounds to sulphate is not clear. However, PIRIE (**52**) has suggested as a working hypothesis the following scheme for animal tissue:

$$\begin{matrix} \text{R.SS.R} \\ \text{or} \\ \text{2R.SCH}_3 \end{matrix} \rightarrow \underset{1}{\text{2R.SH}} \rightarrow \underset{2}{\text{2R.SOH}} \rightarrow \underset{3}{\text{R.SH}} + \underset{4}{\text{R.SO}_2\text{H}} \rightarrow \underset{5}{\text{H}_2\text{SO}_3} \rightarrow \text{H}_2\text{SO}_4.$$

He says that "of these processes the first and second are probably enzymatic, the third proceeds spontaneously at body pH, the fourth is enzymatic and the fifth need not necessarily be so."

The experiments reported show that when citrus fruits are treated with sulphur dust reactions appear to be similar in certain respects to those exhibited in sulphur metabolism when various sulphur compounds are used in the diet of animals or man, or added to cultures of animal tissue; when fungi and bacteria are supplied with sulphur gases, or when sulphur is incorporated in the substrate; and when sulphur is used in the nutrition of higher plants, or when sulphur gases are released into the atmosphere about such plants. Although the literature is far too extensive for review here, the eventual end product of sulphur metabolism appears to be SO_4^{--}.

Summary

1. The writer shows that the data of several investigators indicate that the rate of volatilization of elemental sulphur increases logarithmically with temperature.

2. Radioactive elemental sulphur dusted on lemon fruit penetrated the peel to a depth of 240 μ when the fruit was incubated six hours at 140° F.

3. Citrus fruits dusted with elemental sulphur and incubated at temperatures of 90° to 135° F produce H_2S and SO_2 gases and other volatile constituents. Under aerobic conditions, when both gases are absorbed and assayed simultaneously, the total amount of H_2S and SO_2 produced increases with temperature. Fruits which have not been dusted with sulphur do not produce these gases. Under anaerobic conditions, the rate of production of H_2S and SO_2, declines at increasing temperatures until 135° is reached, and then the rate of H_2S production is increased. Greater quantities of H_2S are produced in late winter and in early spring months than at other times of the year, thus correlating roughly with the glutathione content of the fruit peel.

4. Sulphur-dusted citrus fruits have higher percentages of SO_4 in the peel and peel sap than nondusted fruits. The peel of fruits burned by sulphur while on the tree contains higher percentages of sulphur as SO_4 than that of unburned fruit. Also, the peel from the burned side of the fruit contains higher percentages of sulphur as SO_4 than that from the unburned side. Under some conditions there appears to be a slight but irregular increase in the percentage of sulphur as SO_4 in the peel of sulphur-dusted fruit with increasing temperatures.

5. At the critical peel temperature range at which lemons are injured by elemental sulphur, there is a decrease in pH of peel extracts with increase in temperature from 105° to 122° F. The decrease in pH was in-

terpreted to indicate the formation of H_2SO_4. The pH values attained were often lower than those of the isoelectric points of proteins of the juice.

6. Fruits treated with H_2S gas, when incubated, produce SO_2 gas; such fruits show higher percentages of SO_4 in the peel than untreated controls. The pH of peel extracts of treated fruit is lowered more than that of non-treated controls.

7. Fruits treated with SO_2 gas during incubation showed higher percentages of SO_4 and lower pH values in the peel than untreated controls.

8. One fourth of the glutathione content (reduced form) of the peel roughly accounted for the H_2S produced by elemental sulphur. Under aerobic conditions the glutathione content (reduced form) was lowered by sulphur dusting, but under anaerobic conditions it was increased. Vitamin-C content did not change with sulphur-treatment. Oxidation-reduction potentials were lower in both nondusted and sulphur-dusted, incubated fruit than in extracts of fresh peel.

9. Incubation at increasing temperatures increased the loss of weight of sulphur-dusted fruit over that of nondusted controls in the critical temperature range, but at higher temperatures the relationship was reversed.

10. The loss of weight by sulphur-dusted and incubated fruit is due to loss of water, H_2S, SO_2, and other unidentified gases; and to loss of an acidic white or yellow exudate (red when in sizable quantities) composed of sugars, inorganic and organic acids, pentosans, probably humin compounds, minerals, ammonia, tryptophane, glutamine, and some unidentified substances. Some mineral constituents of the peel were made more soluble by sulphur-dust and incubation treatment; some minor elements were made less soluble.

11. Preheating fruit to high temperatures prior to sulphur-dust treatment and incubation reduced the production of H_2S and SO_2 gases and the SO_4 content of the peel. When the preheating of citrus fruit was followed by incubation in H_2S, the SO_4 content of the peel was reduced, and when preheating of the fruit was followed by incubation in SO_2, H_2S production and SO_4 formation in the peel were reduced. Catalase of the peel was inactivated by sulphur-dust treatment and incubation.

12. Elemental sulphur dust appears to lower the critical temperature at which citrus fruits are injured by absorption of sunlight. The principal factors influencing this temperature in the external environment are: (*a*) intensity of sun radiation, (*b*) temperature of the air, (*c*) vapor density of the air. Injury occurs when the outgoing energy does not balance the incoming energy at a point below the critical temperature. Air temperature and vapor density regulate the rate of outgoing energy.

The writer is indebted to Dr. A. M. Boyce for suggesting the problem, and to the Texas Gulf Sulphur Co. for previous grants in aid which made the successful initiation of this study possible. He also gratefully acknowledges his indebtedness to the following: to Dr. E. T. Bartholomew for refractometer determination of soluble solids; to Dr. L. J. Klotz for collabora-

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THE INFLUENCE OF MINERAL DEFICIENCY ON VEGETATIVE GROWTH, FLOWER AND FRUIT PRODUCTION, AND MINERAL COMPOSITION OF THE PEANUT PLANT[1]

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(WITH FOUR FIGURES)

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Introduction

The peanut plant, *Arachis hypogaea* L., is one of the few plants in which the ovary of the aerial flower must be transferred by a gynophore to a subterranean position before maturation of the fruit will normally occur. The morphology and anatomy of the peanut plant as described by PETTIT (**16**), WALDRON (**23**), REED (**17**), and JACOBS (**11**) indicate that the gynophore has a stem-like anatomy and a root-like behaviour. Pettit (1895)

FIG. 1. Gynophore with root-hair-like epidermal outgrowths.

reported that the hypogeal portion of the gynophore forms root-hair-like epidermal outgrowths and suggested that the organ was a part of the absorptive system since few root hairs were found on the root of the plant. Waldron also assumed that the hairs on the gynophore might absorb water and nutrients, while MOHAMMAD, *et al.*, (**15**) and Reed considered absorption by the hairs to be of little importance. The authors have observed the epidermal outgrowths of the gynophore on field grown plants, and at various periods of the year when that organ developed in solution, sand and/or soil under greenhouse conditions, figure 1.

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[2] A part of this paper was presented before the American Society of Agronomy in Columbus, Ohio, 1946.

At the initiation of the present study the literature offered little information on the nutrition of the peanut. VAN DER WOLK (**21**) observed that water and darkness were essential to fructification, and that a soil extract was beneficial. SHIBUYA (**18**) reported that only the first two factors are necessary. BURKHART and COLLINS (**6**) demonstrated the uptake of lithium by the fruiting organs and reported that fruit quality was benefited by the presence of calcium in the fruiting medium.

BRADY, *et al.* (**4**), from studies initiated concurrent with and since the present investigation, reported a significant increase in fruit filling when a single calcium salt was added to the fruiting medium. Subsequent work by HARRIS (**10**) showed fructification to be negligible when a minus calcium nutrient solution was applied to the fruiting medium. Results of MEHLICH, *et al.*, (**14**) also indicate that fruit filling is related to the available external calcium supply. THORNTON and BROADBENT (**20**) demonstrated the uptake and movement of isotopic nitrogen from the fruiting organs to the vegetative portions of the plant. Recent work with radioactive calcium by BLEDSOE, *et al.* (**3**) indicates that the need of an external supply of calcium by the developing fruit might be the result of poor translocation of that ion from the plant to the fruit.

This report deals with the results of a study conducted in 1945 relative to the necessity of an external supply of nutrients to the fruiting organ for normal fructification and to an evaluation of salt movement from the fruiting organs to the vegetative tissue when differential nutrient treatments were applied to the root medium of the peanut plant.

Experimental

Peanut seeds of the Dixie Runner variety were placed in moist chambers to germinate on April 11, 1945. After five days the most vigorously germinating seeds were selected and single seedlings were transferred to one-gallon jugs filled with acid-water-washed sand which had been inoculated with Nitragen E Culture. When the plants were about 5 cm. high, selected containers with the more uniform individuals were placed in boxes of sand. The method of isolating the rooting and fruiting media, the basic and supplementary nutrient solutions used, and their method of application have already been described (**2**). The pH of all solutions was adjusted to approximately 5.5.

The complete nutrient solution was added to the fruiting medium of 24 plants throughout the experiment, while differential nutrient treatments were started on the roots of the plants after they had received the complete solution for 80 days. At that time a mean of 43 gynophores per plant had penetrated the sand of the fruiting medium. The plants were then divided into eight groups of three replicates of single plants and the following nutrient treatments, with concentrations modified as reported previously (**2**), were begun on the rooting media: complete, minus phosphorus,

minus potassium, minus calcium, minus magnesium, minus sulphur, minus micro-nutrients, and distilled water. Plants which received the complete solution in root and fruiting media throughout the experiment are referred to hereafter as control plants.

Each of three boxes of sand held eight glass jugs with single plants. The eight nutrient treatments on the rooting media were randomized and each treatment occurred once to a single plant in each box. A fourth box contained the ninth group of three jugs of single plants which received the complete nutrient solution on the roots, and distilled water on the fruiting medium throughout the experiment.

The glass jugs, which held the roots of the plants, were originally filled with sand to within 1 inch of the tops. To allow for root growth, portions of the sand were removed April 24 and June 8 by carefully washing it through the drain holes of the jugs. Since the plants made satisfactory growth after each removal of sand it is assumed that the roots were little affected by the operation. The top of each glass jug was placed $1\frac{1}{2}$ to 2 inches above the surface of the sand in the boxes and drainage holes in the bottom of the latter permitted the escape of any excess rain water. Thus, the possibility of nutrients from the fruiting medium contaminating the solutions applied to the roots of the plant within the glass jugs was eliminated. A string mesh across the tops of the boxes prevented the branches and foliage from touching the fruiting medium.

During the course of the experiment, flowers of all plants were counted daily. Since the peanut flower is ephemeral and wilts after a few hours, an accurate record of flower production is obtained easily.

When the plants were 130 days old and had received the deficient solutions on the roots for 50 days, they were harvested; the entire plant and portions thereof were immediately weighed and subsequently dried at 70° C. Harvesting of plants was accomplished by first carefully flushing the sand from the roots and fruits through the drain holes of their respective containers. The glass jugs were then broken and the roots were washed free of sand. This permitted a complete recovery of all fruit and essentially the entire root system of the plant.

The middle leaves of lateral branches and the following stages of fruit development were selected from each plant for mineral analysis: (1) gynophores prior to entering the sand of the fruiting medium, (2) gynophores in sand with slight basal enlargement, and (3) shells of mature fruit. Samples for mineral analysis (phosphorus, potassium, magnesium and calcium) were ashed for four hours in an electric muffle at 500° C. Total nitrogen was determined on separate samples. Nitrogen, phosphorus and calcium were determined by the Official Method of Analysis (**1**), while magnesium was determined by the method of DROSDOFF and NEARPASS (**8**), and potassium by the sodium-cobaltinitrite method of BROWN, ROBINSON and BROWNING (**5**).

TABLE I

THE MEAN GREEN WEIGHT, FLOWERS AND GYNOPHORES PER PLANT OF THE PEANUT AS AFFECTED BY NUTRIENT TREATMENT. ROOTS OF PLANTS GROWN IN COMPLETE SOLUTIONS TO JULY 1 (80 DAYS) AND DEFICIENT SOLUTIONS TO AUGUST 20 (50 DAYS).

NUTRIENT TREATMENT		MEAN GREEN WEIGHT			FLOWER PRODUCTION AT 10-DAY INTERVALS AFTER DEFICIENT SOLUTIONS APPLIED. JULY 1 TO AUGUST 20 (50 DAYS).					MEAN TOTAL FLOWERS July 1 to Aug. 20	MEAN TOTAL GYNOPHORES July 1 to Aug. 20
ROOT ZONE	FRUIT ZONE	TOP (*gms.*)	ROOT (*gms.*)	TOTAL (*gms.*)	July 10	July 20	July 30	Aug. 9	Aug. 19		
Complete*	Complete*	770	103	873	109	84	100	87	96	476	325
Complete	D. Water	848	135	983	114	92	104	94	185	589	447
D. Water	Complete	138	51	189	44	2	9	17	14	86	35
− K	"	484	83	567	100	83	78	50	15	326	180
− P	"	337	73	410	81	81	91	47	24	324	203
− Ca	"	241	49	290	115	78	46	2	0	241	138
− Mg	"	449	77	526	107	109	112	60	55	443	270
− S	"	466	61	527	64	52	61	61	59	297	200
− Micro Nutrients	"	293	32	325	54	64	33	15	17	183	75
L.S.D. 5%		124	29	148						179	125
L.S.D. 1%		170	40	204						246	172

* Referred to in text as control treatment.

TABLE II

MEAN NUMBER AND MEAN PER CENT. PER PEANUT PLANT OF FLOWERS, GYNOPHORES, AND FRUITS AS AFFECTED BY NUTRIENT TREATMENT. ROOTS OF PLANTS GROWN IN COMPLETE SOLUTIONS TO JULY 1 (80 DAYS) AND DEFICIENT SOLUTIONS TO AUGUST 20 (50 DAYS)

NUTRIENT TREATMENT		MEAN FLOWERS	MEAN GYNOPHORES	MEAN SOUND MATURE FRUIT	FLOWERS PRODUCING GYNOPHORES %	FLOWERS PRODUCING FRUIT %	GYNOPHORES ENTERING FRUITING MEDIUM %	GYNOPHORES IN FRUITING MEDIUM PRODUCING FRUIT %
ROOT ZONE	FRUIT ZONE							
C†	C†	644	378	59.3	58.7	9.2	64.3	24.4
C*	O**	758	495	1.5	65.3	0.2	44.2	0.7
O	C	250	74	3.7	29.6	1.5	78.4	6.4
– K	C	481	229	41.5	47.6	8.6	76.8	23.6
– P	C	475	243	29.7	51.2	6.3	72.8	16.8
– Ca	C	392	179	21.0	45.7	5.4	69.8	16.8
– Mg	C.	583	312	13.7	53.5	2.3	52.9	8.3
– S	C	423	236	37.7	55.8	8.9	66.9	23.9
– Micro-Nutrients	C	324	122	1.7	37.7	0.5	53.3	2.6
L.S.D. 5%		164	130					
L.S.D. 1%		225	179					

* = Complete nutrient solution.
** = Distilled water.
† = Referred to in text as control treatment.

FIG. 2. Peanut plant which received a complete solution in the rooting medium and distilled water in the fruiting medium.

TABLE III

THE MINERAL COMPOSITION OF PEANUT LEAVES, GYNOPHORES BEFORE AND AFTER ENTERING SAND OF FRUITING MEDIUM AND SHELLS OF MATURE FRUIT
MEAN PERCENTAGE COMPOSITION—OVEN DRY BASIS

NUTRIENT TREATMENT		NITROGEN				CALCIUM			
Rooting medium	Fruiting medium	Middle leaves	Young gyno.*	Gyno. in sand	Shells of fruit	Middle leaves	Young gyno.	Gyno. in sand	Shells of fruit
Complete**	Complete**	4.29	3.08	2.96	2.35	2.12	0.36	0.48	0.19
Complete	Water	4.02	3.26	3.08	.....	1.91	0.41	0.39	0.13
Water	Complete	3.85	.....	3.36	.....	0.92	0.49	0.39	0.09
– K	"	3.84	3.53	3.27	2.41	3.23	0.56	0.44	0.14
– P	"	3.08	2.74	2.87	2.39	1.89	0.48	0.37	0.13
– Ca	"	3.88	2.51	2.84	2.22	0.71	0.16	0.25	0.13
– Mg	"	3.81	2.68	2.71	2.21	1.85	0.76	0.63	0.12
– S	"	4.33	3.16	2.95	2.41	2.14	0.37	0.44	0.17

NUTRIENT TREATMENT		MAGNESIUM				PHOSPHORUS				POTASSIUM			
Rooting medium	Fruiting medium	Middle leaves	Young gyno.	Gyno. in sand	Shells of fruit	Middle leaves	Young gyno.	Gyno. in sand	Shells of fruit	Middle leaves	Young gyno.	Gyno. in sand	Shells of fruit
Complete**	Complete**	0.72	0.26	0.21	0.20	0.26	0.31	0.26	0.15	4.24	3.68	4.56	2.37
Complete	Water	0.74	0.23	0.17	0.20	0.22	0.30	0.26	0.31	3.91	3.50	3.23	1.70
Water	Complete	0.29	0.13	0.18	0.09	0.22	0.31	0.29	0.17	2.47	1.42	1.17	0.47
– K	"	1.38	0.35	0.34	0.17	0.55	0.43	0.46	0.17	0.21	0.68	1.49	1.41
– P	"	0.50	0.20	0.17	0.16	0.08	0.15	0.11	0.06	3.63	2.37	2.72	1.46
– Ca	"	0.71	0.43	0.28	0.21	0.33	0.35	0.36	0.19	6.44	4.80	5.30	2.99
– Mg	"	0.06	0.07	0.09	0.08	0.25	0.42	0.37	0.13	4.88	3.46	3.44	2.20
– S	"	0.50	0.26	0.28	0.25	0.26	0.29	0.25	0.12	3.36	3.05	4.11	2.22

* Gynophores.
** Referred to in text as control treatment.

Results

The necessity of an external supply of nutrients in both the rooting and fruiting media of the peanut plant for optimum growth and fruit production is shown in tables I, II, and III. Without exception it was found that plants supplied with the complete solution in the rooting medium, irrespective of the nature of the fruiting medium, notably surpassed the plants of all other cultures not only in the total amount of vegetative growth, flower and gynophore production, but also in the character of growth and general appearance of the plants. However, the absence of an external supply of nutrients in the fruiting medium resulted in a highly significant decrease in fructification. Fruit development was negligible when distilled water only was used in the fruiting medium (fig. 2) in contrast to the number of fruit produced by the control plants (fig. 3).

FIG. 3. Peanut plant which received a complete nutrient solution in the rooting and fruiting media.

The former plants had a favorable appearance and exceeded the control plants in vegetative vigor and in the number of gynophores produced. A mean number of 219 gynophores per plant had entered the fruiting medium, but there was limited hypogeal development beyond the initial stage of enlargement after penetrating the sand. This indicates the necessity of some nutrients in the fruiting medium of the peanut plant for favorable fructification.

The necessity of a nutrient balance in the rooting medium is clearly indicated by the results from the deficient cultures. Vegetative growth

and mineral composition of plants were obviously affected by the withholding of any ion or ions from the external supply of the roots when the fruiting organs were supplied the complete solution. Withholding all nutrients from the roots after July 1, resulted in the smallest plants in the experiment. Highly significant differences are demonstrable between data of the latter and control plants in all characters used as criteria for vegetative vigor and fruitfulness. When only distilled water was applied to the roots, flowering practically ceased after four days and slight vegetative growth was made thereafter. The extreme retarded growth of previously vigorously growing plants in such a short period after all nutrients were withdrawn from the roots and the limited amount of growth thereafter when there were many gynophores and some fruits developing in the medium supplied

FIG. 4. Peanut plant which had micronutrients withheld from the roots after 80 days while the fruiting medium received a complete solution throughout the experiment.

the complete nutrient solution leads to the conclusion that there was a limited movement of minerals from the fruiting organs to the vegetative tissue of the plants under conditions of this experiment. Likewise, the rapid development of foliar deficiency symptoms for mineral elements which resulted from the deletion of the external supply to the roots when the fruiting media were supplied the complete solution further supports that conclusion.

Plants were grown under conditions conducive to fruitfulness and during the period when peanuts are usually grown in the field. The deficient solutions were applied to the roots of the plants during the most active period of flowering. Many gynophores produced in August by plants

grown on the complete solution were from stems which extended over the sides of the culture boxes. The yields reported for the control plants indicate that the rooting and fruiting media of the cultures had a favorable supplying power for nutrients. All plants were harvested approximately 20 days short of the usual growth period because of the severe nutrient deficient condition of some plants. As a result, the control plants had many immature fruits which resulted in less difference between yields of control and deficient cultures than would have occurred had plants been harvested at a later date.

Summarization of data from tables I and II shows that when the complete solution was supplied to the fruiting media continuously and to the roots of plants for an 80-day period followed by a 50-day treatment to the roots with nutrient deficient solutions, it resulted in a statistically significant decrease between data of the latter and that of the control plants in the following characters: (1) the mean green weight of all cultures, (2) the mean number of flowers of all except the minus potassium and minus magnesium cultures, (3) the mean number of gynophores of all except the minus magnesium cultures, and (4) the mean number of fruits for all except the minus potassium cultures.

Figures of mineral composition (table III) represent the mean of duplicate analyses of each of three plants. The mineral composition of middle leaves from lateral branches is assumed to be representative of the nutritional status of the vegetative portion of the plants. Likewise, the mineral content of gynophores before and after they entered the sand of the fruiting zone, and shells of mature fruit indicate the influence of nutrient treatment on the mineral composition of those organs.

The withholding of all nutrients from the roots of the plant in general resulted in a much lower percentage mineral content of plant parts analyzed than did the omission of nutrients from the fruiting zone of the plant. Withholding an element from the roots resulted in a decreased percentage of that element in the leaves and all fruiting organs. However, there was a tendency for the calcium and especially the potassium concentrations in the gynophores to increase after entering the fruiting zone supplied with the complete nutrient solution. This occurred when the complete solution was applied to both rooting and fruiting media and when the minus potassium, minus calcium, and minus sulphur solutions were applied to the roots of the plants. There was a slight decrease in the concentration of all minerals in the gynophores after entering sand to which only distilled water had been applied. When all nutrients were withheld from the roots of the plant after 80 days, it is questionable if the data of mineral content of the gynophores are comparable because thereafter very few gynophores entered the sand of the fruiting medium.

When the roots of the plants received the minus phosphorus or minus magnesium solution, the phosphorus and magnesium concentrations, re-

spectively, of the gynophores did not increase after those organs entered the zone supplied the complete solution. The shells of mature fruit likewise had a very low concentration of those ions. Thus, the data suggest that after the gynophore enters the fruiting medium, it has a greater absorptive intensity for potassium and calcium than for phosphorus or magnesium, which might indicate a greater uptake of potassium and calcium from the external supply in that zone.

Mineral deficiency symptoms

Reports of symptoms of mineral nutrient deficiencies other than for the young peanut plant do not occur in the literature (**6**). For that reason, a description of nutrient deficiency symptoms of older plants as they occurred in this investigation will be recorded briefly with appropriate comments.

The early stages of leaf symptoms under conditions of phosphorus deficiency became evident within two weeks after phosphorus was withheld from the roots (**6**). Top growth was greatly restricted and in the later stages there was premature defoliation of the yellow basal leaves, but stem discoloration did not occur (**6**).

Three weeks after the potassium deficient solution was applied to the roots of the plant the foliage became a darker green in color. In later stages there was some abscission of lower leaves, but marginal necrosis of leaflets was very limited. After 40 days the terminal portion of stems and adjacent petioles became distinctly red, then brown, which was followed by death of the tissue. Fructification of the potassium deficient plants was significantly greater than plants of the other deficient cultures.

Magnesium deficiency as previously reported by BLEDSOE, *et al.*, (**2**) did not show the orange coloration of leaflet margins reported by BURKHART and COLLINS (**6**). It is interesting that while flower and gynophore production of the minus magnesium cultures was not significantly less than that of the control plants, fructification was significantly decreased. Data in table II and results reported by SOMMER and BAXTER (**19**) indicate the importance of magnesium in nut production.

Within two weeks after treatments were started, symptoms of calcium deficiency became apparent by restricted growth and the appearance of small interveinal brown pitted areas accompanied by marginal chlorosis on the upper surface of fully developed leaflets proximal to the growing tips. At 27 days the leaflets at the tips were very small, chlorotic, crinkled and distorted. Pitted areas were then present on both leaf surfaces and had coalesced to form numerous larger necrotic spots which resulted in a bronze leaf color. Basal stem cracks and die-back of the affected shoots occurred in the later stages. Roots of plants were stunted and decomposing when harvested. Blossoming had decreased sharply within 10 days and was entirely suppressed prior to termination of the experiment. Gynophore and fruit production was also significantly less than that of the control plants. Re-

sults indicate the necessity of an ample supply of calcium in the rooting medium of the peanut plant irrespective of the nature of the fruiting medium.

External deficiency symptoms for sulphur were not recognizable. Shoot and root growth was significantly decreased and nodulation was less than that of the control plants. Flower production remained rather constant throughout the period that the plants received the sulphur deficient solution on the roots.

The omission of the micro-nutrients (Cu, Mn, Zn, B and Mo) from the roots of the plants after 80 days resulted in a highly significant decrease in all characters used as criteria for vegetative vigor and fruitfulness, figure 4. Flowers were pale yellow in color and a high percentage failed to produce gynophores. Interveinal chlorosis of the terminal leaflets appeared at 27 days and all new growth of lateral branches was affected as age advanced. The failure of the gynophores to supply micro-nutrients to the plants shoots in quantities sufficient for growth indicates that the absorption by those organs was of a low order under conditions of this experiment.

The withholding of all nutrients from the roots of the plant at 80 days greatly restricted top growth and blossoming within a 10-day period. That was followed by early maturation, defoliation of some basal leaves, and the appearance of cracks in the basal portion of stems. Necrotic spots appeared on the terminal leaflets, and the meristematic region was affected in a similar manner to the plants grown on the minus calcium solution. Chlorotic effects occurred on the young growth and had spread progressively toward the older leaves when the experiment was terminated.

Discussion

In this investigation, those plants supplied with a deficient solution to the root medium and a complete solution to the fruit medium were considered as deficient cultures. The reserve of the deficient ion in the foliage, absorbed during the preliminary period when the plants were grown on the complete solution, and the redistribution of the reserve within the plant after the external supply of the ion had become deficient to the root system probably in part accounts for the differences in quantity of fruit produced by plants grown on a given deficient solution.

Results indicate that the root is the primary absorbing organ of the peanut plant and an inadequate supply of nutrients in that region will have a deleterious effect on both vegetative growth and fructification. The rapid development of foliar deficiency symptoms after the deficient solutions were applied to the roots of the plants, when the fruiting medium was supplied the complete solution, indicates that if elements were absorbed by the fruiting organs from the external supply in the fruiting zone, then there was a small amount translocated to the plant shoot. In some instances it appeared as if the developing fruit drew on the reserves of the leaves, thus accelerating breakdown of the vegetative tissue. This is consistent with the general

view that the mineral requirements of the fruit are satisfied at the expense of the vegetative tissue if the external supply falls short of supplying the needs of both. However, it has been demonstrated by HAMNER (**9**), LOEHWING (**12**), and WADLEIGH (**22**) that the lack of balance may accentuate a given deficiency or be more detrimental to growth than generally realized.

It is assumed that absorption of some elements would occur by an organ having the hypogeal structure of the gynophore (fig. 1). BURKHART and COLLINS (**6**) demonstrated the uptake of lithium by the fruiting organ and its distribution within the plant. The nutritional status of the plant might have an influence on the absorption by the gynophores as shown by the work of THORNTON and BROADBENT (**20**), where the absorption of N^{15} was negligible when an ample supply of nitrogen was available to the roots, but it was considerably increased when the roots were given a low nitrogen supply. However, in all cases the amount of N^{15} absorbed by the gynophores represented only a small part of the total nitrogen of the plant. HARRIS (**10**) reported the uptake of radioactive phosphorus and cobalt by the fruiting organs to be insignificant when compared with that absorbed by the roots of the plants. The fruiting organs received a much greater amount of those ions when applied to the root medium of the plant than when the fruiting organs were in direct contact with the radioactive substances.

There are no data available on the ratio of surface of roots to hypogeal portion of gynophores. In all probability the absorptive area of the latter would represent only a small portion of the former and consequently the total amount of absorption by the fruiting organs would be expected to be relatively small as compared to the root system. Furthermore, some investigators assume that absorption by the fruiting organs occurs primarily during the early stages of fruit development, which, if true, would further limit the absorption period of those organs.

Obviously, the results of this investigation give no indication of the ionic requirements for fructification. This, of necessity, follows from the nature of the experimental set-up, which does not permit a measurement or even an approximation of the external ions needed, the amounts or the stage of fruit development when such are essential. However, data of mineral composition (table III) suggest that the fruiting organs had a greater absorption intensity for potassium and possibly calcium from the external supply in the fruiting medium than for phosphorus or magnesium. These results also show that the nutrient deficient solutions had a greater effect on the potassium content of fruiting organs than that of other elements determined. Results by BURKHART and COLLINS (**6**) and BRADY, *et al.*, (**4**) indicate that the known antagonism between potassium and calcium may come into play when the fruiting medium has a low available calcium supply. It is probable that an excessive potassium absorption by the roots of the plant or by the developing fruit may influence the transport of calcium from the vegetative organs to the fruit or the direct intake of calcium by the fruit.

The importance of an available calcium supply in the fruiting medium for favorable fructification has been emphasized by a number of investigators (**4, 6, 10, 14**). BLEDSOE, *et al.*, (**3**) recently reported that when radioactive calcium was administered to the fruiting medium there was a very active absorption of Ca^{45} by gynophores, shells and seed of fruit as compared with the small amount of absorption by those organs when Ca^{45} was supplied to the root of the plant. In the latter case, the young gynophore had a relatively high concentration of Ca^{45} but the concentration per unit of dry weight decreased as that organ developed and the shells of fully developed green fruit contained only a small concentration of labeled calcium while never more than a trace could be detected in the seed. Thus, it appears that the transport of calcium from the plant to the rapidly growing gynophore is sufficient for its elongation but probably inadequate for conditions of protoplasm, wall formation, etc., required in fruit growth and development. The relative immobility of calcium and some other ions as reported by MASON and PHILLIS (**13**) will be recalled in this connection.

The available calcium supply in the fruiting medium has been demonstrated to have a greater influence on fruit filling than the availability of any other known element. However, from data of table II, those reported by other investigators (**4, 7, 10,**) and unpublished results by the authors, it appears obvious that factors other than the available calcium supply in the fruiting medium are important in fructification. The number of well developed fruit even with the best known nutrient treatments represents a small per cent. of the gynophores which enter the fruiting medium. Whether that failure of development is related to organic or inorganic supply is as yet undetermined. Whatever the role of the external supply of calcium in fruit development may be, it would appear that its role would be also other than that of merely adding calcium to the seed.

Summary

Peanut plants of the Dixie Runner variety were grown in sand culture with a complete nutrient solution applied to the isolated fruiting media, while the roots of the plants received various deficient nutrient solutions following 80 days of growth on a complete solution. Nutrient deficiency symptoms which developed under the above conditions are described. Vegetative growth, fruit production, and mineral composition of plant parts were adversely affected when any macro-element was withheld from the roots of plants after 80 days. Fruit production was negligible when the roots received the complete solution, and distilled water was added to the fruiting zone of the plant. Mineral composition of gynophores before and after entering that zone supplied with the complete solution suggests that those organs have a greater absorptive intensity for potassium and calcium from the external supply in the fruiting medium than for phosphorus or magnesium. Results show that the root is the primary absorbing organ of the

peanut plant and the necessity of a nutrient balance in the root zone for optimum growth and fruit production is emphasized. In no case was the absorption of an element by the gynophores sufficient to offset the appearance of nutrient deficiency symptoms of that element when it was omitted from the roots of the plants.

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CORRELATION BETWEEN LEAF COLOR, LEAF NITROGEN CONTENT, AND GROWTH OF APPLE, PEACH, AND GRAPE PLANTS

WESLEY P. JUDKINS AND I. W. WANDER

(WITH THREE FIGURES)

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Numerous articles and bulletins have been published in recent years describing various foliar symptoms of nutrient deficiency. A rather comprehensive compilation of this material has been assembled in book form by HAMBRIDGE (**5**).

Among these papers are many dealing with nitrogen as a nutrient element and its importance in increasing the vigor of plants and the green color of the foliage. Critical evaluation of this green color and the quantitative determinations of its relationship to plant growth have received much less consideration. COMPTON *et al.* (**3**) reported on the correlation between leaf color of McIntosh apple trees and the amount of nitrogen fertilizer which was applied. As a result of this study, color charts were developed which could be used as an indication of the amount of nitrogen fertilizer which should be applied to McIntosh apple trees to insure satisfactory growth and yields.

The development of leaf color standards marks a progressive step in the application of experimental results to fertilization practices in the field. However, each technician or field man will vary in his ability to match accurately the leaf sample with colored plates since there is known to be a high incidence of red-green color blindness in men. If leaf color is to become a useful tool in determining the amount of fertilizer which is to be applied to plants, a better method of color evaluation should be developed.

The tests being reported in this paper were initiated to determine the value of a photoelectric reflection meter as a means of measuring leaf color. This instrument does not measure color directly but does give an accurate numerical rating of the luminous reflectance of any surface. Luminous reflectance may be described as that attribute of a surface which characterizes it as being of a particular lightness or darkness. Variations in the intensity of green pigmentation of leaves may be evaluated by this reflectometer and the results serve as an indirect measure of leaf color.

The reflection meter used in these tests was model 610 manufactured by the Photovolt Corporation of New York City. This instrument is essentially a photoelectric cell in conjunction with colored glass filters and a galvanometer with a scale calibrated from 0 to 100. A green tristimulus filter was used in the tests reported in this paper. Such equipment has

been used for a number of years in the ceramic and paint industries for determining color, as well as color changes during the weathering of paints.

The instrument is calibrated on the basis of a freshly prepared magnesium oxide surface having a value of 100. The reflectance readings near 100 refer to an essentially white surface. Readings in the lower ranges of the galvanometer scale indicate dark surfaces with low reflectance values.

The principal objective of these experiments was to determine the value of the reflectometer as a method of measuring leaf color. A second objective was to secure data on the relationship between leaf color (reflectance), leaf nitrogen, and the growth of several fruit producing plants.

A previous report of JUDKINS (**6**) describes the use of this equipment in connection with peach trees growing under orchard conditions.

Materials and methods

One-year-old plants of Cortland, Delicious and Stayman Winesap apple, Concord and Delaware grape, and Halehaven peach were planted in early April 1946, in screened ($\frac{1}{8}$ to $\frac{3}{8}$ inch) washed gravel in 3-gallon glazed crocks. Moisture and nutrients were supplied three times daily by pumping a nutrient solution into the gravel from the bottom, until the surface was flooded. The solution was then allowed to drain by gravity back into the carboys which served as reservoirs.

The basic nutrient solution to which various amounts of nitrogen were added contained the following chemicals at the indicated molar concentrations: KH_2PO_4 at .0022 M., $CaCl_2$ at .0045 M., $MgSo_4$ at .0022 M., $MnSO_4$ at .00005 M., and H_3BO_3 at .00005 M. Iron was supplied by adding one milliliter of a 0.5% solution of ferric citrate for each liter of solution at the time they were changed each week. This basic solution is similar to those used by BLAKE, NIGHTINGALE, and DAVIDSON (**1**) and by WALTMAN (**9**). The nitrogen concentrations of the nutrient solutions were varied according to the following schedule.

The apple trees and grape vines were supplied with nitrogen at 0, 15, 60 and 160 parts per million. Four plants of each of the three varieties of apples were grown under each nitrogen level, making a total of 48 trees under test. Four plants of each of the two varieties of grapes were likewise grown under each of the four nitrogen levels, making a total of 32 grape vines.

The Halehaven peach trees were supplied with nitrogen at 0, 10, 30, 90, and 270 p.p.m. Four trees were grown under each nitrogen level making a total of 20 trees.

In all treatments the nitrogen was supplied in rates of 4 parts of $NaNO_3$ to 1 part $(NH_4)_2\ SO_4$ as a means of maintaining the nutrient solution at slightly below pH 6.0, in accordance with the results reported by TRELEASE and TRELEASE (**8**).

Leaf samples were collected on June 25, July 24, and September 17 for reflectance and total nitrogen determinations. An average of eight leaves

were removed from each plant at each sampling date. These leaves were selected from the center of the current season's shoot growth. Reflectance was determined on a random selection of 20 leaves from each composite

TABLE I

LEAF NITROGEN CONTENT, LEAF REFLECTANCE, AND DRY WEIGHT OF SHOOTS OF PEACH, GRAPE, AND APPLE PLANTS GROWING IN GRAVEL SUPPLIED WITH NUTRIENT SOLUTIONS CONTAINING DIFFERENT CONCENTRATION OF NITROGEN

VARIETY OF FRUIT PLANT. NITROGEN IN NUTRIENT SOLUTION, P.P.M. CORRELATION COEFFICIENT	LEAF N., % DRY WT. 7/24/46	LEAF REFLECTANCE 7/24/46	DRY WT. OF SHOOTS 10/20/46
		%	*gms.*
Halehaven peach			
0 p.p.m. N	1.77	14.5	4.49
10 p.p.m. N	1.88	14.5	5.11
30 p.p.m. N	2.20	12.7	8.11
90 p.p.m. N	2.48	10.6	15.93
270 p.p.m. N	3.42	7.7	25.77
Correlation coefficient with % leaf N	.....	.9845	.9860
Significance of correlation	.....	99 : 1	99 : 1
Concord grape			
0 p.p.m. N	1.75	12.7	5.06
15 p.p.m. N	1.74	13.2	11.38
60 p.p.m. N	2.36	9.0	20.30
160 p.p.m. N	2.63	5.7	24.65
Correlation coefficient with % leaf N	.....	.9966	.9609
Significance of correlation	.....	99 : 1	19 : 1
Delaware grape			
0 p.p.m. N	1.53	12.8	.78
15 p.p.m. N	1.79	10.3	2.23
60 p.p.m. N	2.23	8.0	4.83
160 p.p.m. N	2.62	6.5	15.65
Correlation coefficient % leaf N	.....	.9829	.9241
Significance of correlation	.....	49 : 1	9 : 1
Delicious apple			
0 p.p.m. N	1.30	12.2	6.78
15 p.p.m. N	1.66	9.5	10.72
60 p.p.m. N	2.11	7.0	20.20
160 p.p.m. N	2.52	6.0	19.42
Correlation coefficient with % leaf N	.....	.9785	.9315
Significance of correlation	.....	19 : 1	9 : 1
Cortland apple			
0 p.p.m. N	1.28	12.8	4.49
15 p.p.m. N	1.55	9.8	11.33
60 p.p.m. N	1.90	7.2	23.57
160 p.p.m. N	2.14	7.0	16.29
Correlation coefficient with % leaf N	.....	.9608	.7997
Significance of correlation	.....	19 : 1	None
Stayman Winesap apple			
0 p.p.m. N	1.47	10.0	7.89
15 p.p.m. N	1.66	8.8	11.52
60 p.p.m. N	1.86	7.8	23.30
160 p.p.m. N	2.21	6.8	11.67
Correlation coefficient with % leaf N	.....	.9815	.3057
Significance of correlation	.....	49 : 1	None

sample. The search unit of the reflectometer was placed at approximately the center of the top surface of each leaf.

Total nitrogen of the leaves was determined by the Kjeldahl method after the reflectance values had been secured. The leaf nitrogen results were calculated on a dry weight basis.

In October, at the end of the growing season, the plants were removed from the crocks. All of the current season's shoot growth was cut off, placed in an oven at 105° C, and the dry weight determined. The weight data represent the differences in growth which resulted from the variations in nitrogen content of the nutrient solutions.

Results and discussion

The data on per cent. leaf nitrogen, leaf reflectance, and dry weight of shoots are presented in table I. The leaf nitrogen and reflectance data for July 24 are recorded in this table. The data of June 25 and September 17 are similar to those of July 24 and have not been included in this report.

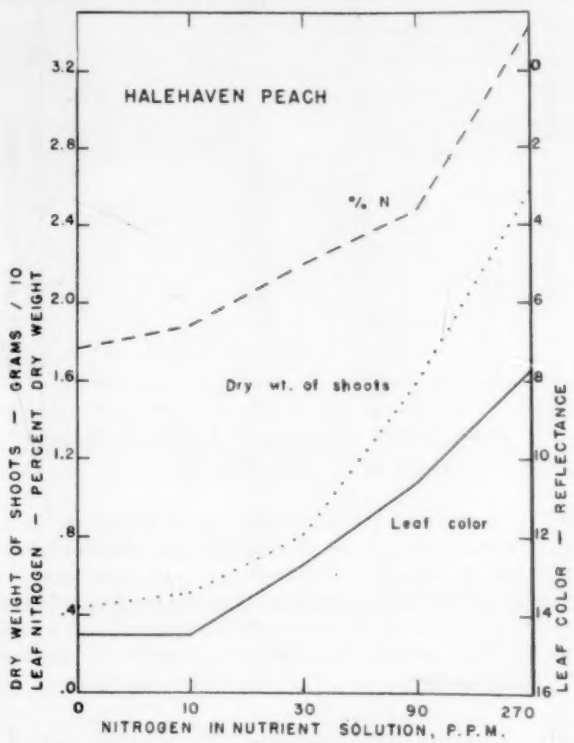

FIG. 1. Leaf nitrogen content, leaf reflectance, and dry weight of shoots of 1-year-old Halehaven peach trees growing in gravel supplied with nutrient solutions containing different concentrations of nitrogen. Leaf nitrogen and reflectance were determined on July 24. Dry weight of shoots was determined on October 20, at the end of the growing season. Correlation coefficients between leaf nitrogen and leaf reflectance, and between leaf nitrogen and dry weight of shoots were very highly significant at odds of greater than 99 to 1.

The data of July 24, which are presented and discussed here, are somewhat more representative of the growth responses of the plants being studied. The results of WANDER (10) and other workers have likewise indicated that late July is the preferred time for taking leaf samples for analysis for nutrient element content.

The per cent. nitrogen, leaf reflectance and dry weight of shoots of the Halehaven peach trees is presented graphically in figure 1. The correlation coefficient between leaf nitrogen and leaf reflectance was very highly significant with odds of greater than 99 to 1.

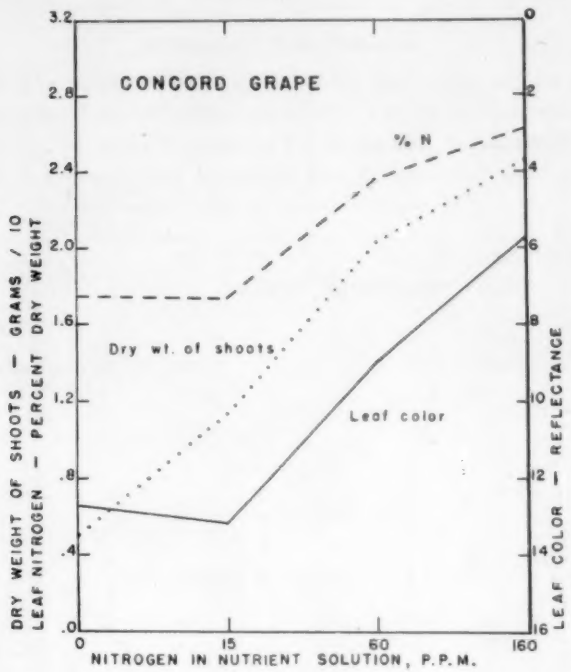

FIG. 2. Leaf nitrogen content, leaf reflectance, and dry weight of shoots of 1-year-old Concord grape vines growing in gravel supplied with nutrient solutions containing different concentrations of nitrogen. Leaf nitrogen and reflectance were determined on July 24. Dry weight of shoots was determined on October 20, at the end of the growing season. Correlation coefficient between leaf nitrogen and leaf reflectance was very highly significant at odds of greater than 99 to 1. Correlation coefficient between leaf nitrogen and dry weight of shoots was highly significant at odds greater than 19 to 1.

The data on leaf reflectance, leaf nitrogen content, and dry weight of shoots of the Concord grape vines were presented graphically in figure 2. In this case the correlation between leaf nitrogen and leaf reflectance is very highly significant with odds of greater than 99 to 1. The correlation between leaf nitrogen and dry weight of shoots is highly significant with odds of greater than 19 to 1.

In the case of Delaware grapes, positive correlations existed between leaf nitrogen and leaf reflectance (odds 49:1), and between leaf nitrogen and dry weight of shoots (odds 9:1). Although the correlations, in these two instances, were less pronounced in the Concord vines, the results are definitely significant.

The results secured with the three varieties of apples are illustrated by the data for Delicious which are presented graphically in figure 3. The

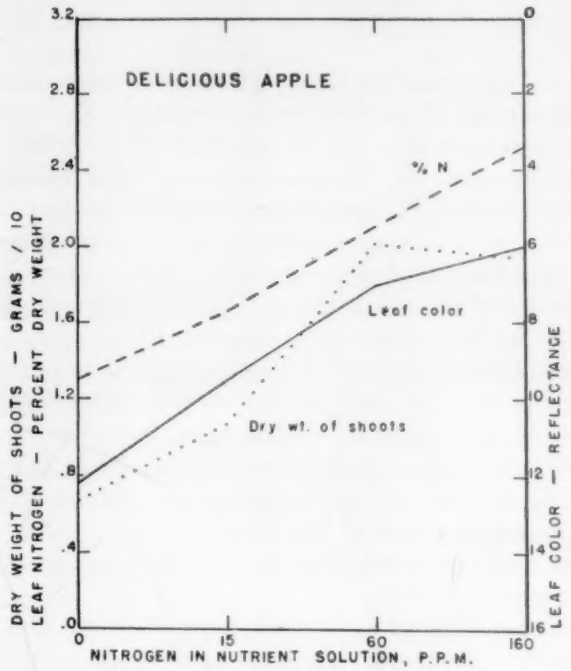

FIG. 3. Leaf nitrogen content, leaf reflectance, and dry weight of shoots of 1-year-old Delicious apple trees growing in gravel supplied with nutrient solutions containing different concentrations of nitrogen. Leaf nitrogen and reflectance were determined on July 24. Dry weight of shoots was determined on October 20, at the end of the growing season. Correlation coefficient between leaf nitrogen and leaf reflectance was highly significant at odds greater than 19 to 1. Correlation coefficient between leaf nitrogen and dry weight of shoots was significant at odds greater than 9 to 1.

correlation between leaf nitrogen and leaf reflectance is highly significant with odds greater than 19 to 1. A similar relationship existed between leaf nitrogen and reflectance in Cortland (odds 19:1), whereas a more significant correlation was found with Stayman Winesap (odds 49:1).

The correlation between leaf nitrogen and dry weight of shoots in the apple trees was more variable than in the other plants studied in this experiment. In only the Delicious variety were the correlations significant (odds 9:1). As can be seen by examining the data in table I, the dry weights of the shoots of Cortland and Stayman Winesap were highest in

the plants which were supplied with the nutrient solution containing 60 p.p.m. of nitrogen, and decreased sharply in the 160 p.p.m. treatments.

A number of workers including BOYNTON and BURRELL (**2**), CULLINAN and BATJER (**4**), and WANDER (**10**) have emphasized the value of leaf nitrogen content as an indication of the nitrogen requirements of apple and peach trees. The results reported in this paper demonstrate that leaf reflectance determinations obtained with the photoelectric reflection meter may be a useful and time saving substitute for nitrogen determinations in estimating the amount of nitrogen fertilizer to be applied to apple, peach, or grape plants.

Plant physiologists may justifiably question the value of leaf color as a means of determining the nitrogen needs of plants. The intensity of this green color is influenced by many factors such as the presence or absence of various nutrient elements, soil moisture supply, and damage by diseases or insects. The situation is further complicated by the fact that interrelationships exist between several nutrient elements which influence the foliar symptoms of deficiencies of these elements. In this connection, SHEAR, CRANE, and MYERS (**7**) have stated that "There are no symptoms the cause of which can be accurately diagnosed visually."

In contrast to the above observations however, it has been repeatedly demonstrated that nitrogen is the nutrient element to which temperate zone fruit plants more often respond. The value of leaf color in planning the nitrogen fertilizer schedule for McIntosh apple orchards has already been reported by COMPTON, GRANVILLE, BOYNTON, and PHILLIPS (**3**). The use of the reflectometer described in this paper appears to offer a more accurate method of leaf color determination than the color charts used by the above named research workers.

Leaf color as an indicator of nitrogen status of plants is useful over the range of "poverty adjustment" up to the point where optimum growth is attained. Beyond this point, in the range of "luxury consumption," variations in leaf color are not significant. Also, as has been indicated previously, the relationship between leaf color and nitrogen is valid only where it is positively known that other nutrient elements are not limiting.

Summary

A method of determining leaf "color" by the use of a photoelectric reflection meter is discussed.

Data are presented which show significant positive correlations between leaf nitrogen content and leaf reflectance of peach, grape, and apple plants growing in gravel supplied with nutrient solutions containing different concentrations of nitrogen. Significant positive correlations were found in the same plants between leaf nitrogen and dry weight of shoots except in the case of Cortland and Stayman Winesap apple.

The results demonstrate that leaf reflectance determinations obtained with the photoelectric reflection meter may be a useful and time saving

substitute for nitrogen determinations in estimating the amount of nitrogen fertilizer to be applied to apple, peach, or grape plants.

There would appear to be no reason why this instrument could not be used to measure the color of other plant parts, such as fruits, where differences in degree of color must be determined.

The reflectometer used in this study appears to be superior to color charts as a means of determining the intensity of the green color of leaves. This instrument eliminates the factor of "human error" which is present when colors are determined by the visual matching technique.

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A MODIFIED CIRCUIT FOR SLIMLINE FLUORESCENT LAMPS FOR PLANT GROWTH CHAMBERS

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Fluorescent lamps were tested several years ago (**1, 2, 3**) as the primary source of visible radiation for plant growth and their use has since been adopted for this purpose by many investigators. Such lamps have the advantages of high luminous efficiency, low temperature, and simplicity of operation. Against these may be ranged the disadvantages of low intensity, possible deficiency in red radiation, multiplicity of wires, and excessive weight of ballast equipment.

For several years the authors have been comparing the growth of soybeans obtained in controlled-environment rooms equipped with carbon-arc and incandescent-filament lamps (**6**) with that produced in similar rooms equipped with fluorescent lamps alone or with additional incandescent-filament lamps. The purpose of these tests has been to find a source of radiant energy consisting principally of fluorescent lamps that would be satisfactory for growing plants. These experiments, which will be reported more fully at another time, have included tests of standard lamps of different colors, tests of experimental lamps containing special phosphors designed for emission of radiant energy, the spectral composition of which might be better suited for growing plants, and investigation of a circuit that avoids the use of conventional ballasts and that simplifies the wiring of the lamps.

The present paper is concerned primarily with this last point. In the course of the experiments a number of investigators became interested in the circuit and some have made similar installations. It is desirable, therefore, to describe the circuit and to call attention to its various advantages and disadvantages.

The earlier installations of fluorescent lamps were mainly the 30-watt, 36-inch type, in daylight or white or a mixture of the two. The introduction of the "slimline" fluorescent lamp of 8-foot length provided a lamp that was capable of illuminating larger areas of bench space with fewer tubes and therefore fewer sockets and less wiring. These lamps are usually energized with a standard inductive ballast that supplies either 100, 200, or 300 milliamperes. The lumen ratings of 4500° white lamps increase from 2100 to 3050 to 3950 as the current is increased.

Since high irradiance is desirable for plant growth the lamps should be energized with the highest practical amperage. This of course has limitations due to the capacity of the lamp and to the excessive size and weight of the ballast. The 200-milliampere ballast is $2\frac{3}{8} \times 3\frac{1}{8} \times 14\frac{3}{8}$ inches and

weighs 14 pounds while the 300-milliampere ballast is $2\frac{5}{8} \times 3\frac{1}{8} \times 19\frac{1}{4}$ inches and weighs 17 pounds. If, for example, one designs a panel of 20 lamps which will uniformly illuminate a bench 6×3 feet he must provide support for 140 pounds of ballast weight if he is using the 200-milliampere ballast, or 170 pounds if he uses the 300-milliampere ballast.

In order to avoid this excessive weight and at the same time to simplify the wiring, a panel of eighteen 8-foot slimline tubes with incandescent lamps serving as ballasts was constructed. This type of ballast has had earlier applications; in fact, it was recommended for a time during the war when standard ballasts were unavailable. Under most circumstances its greater inefficiency than the conventional ballast would preclude its use; but for plant growth it appeared probably that the fluorescent light would require a red supplement, in which event the radiant energy from the incandescent ballast might prove sufficiently useful to compensate for the inefficiency of the circuit.

This panel consisted of eighteen 8-foot slimline lamps mounted on a 48×105 inch sheet of Masonite board with the sockets set as close together as possible. The Masonite panel was supported by a welded angle-iron frame. The lamps and sockets occupied a space $34\frac{1}{2} \times 96$ inches. Since each tube required three 60-watt 115-volt incandescent-filament lamps as a ballast, 54 porcelain sockets were installed in two rows of 27, each row being placed near the edge of the board, parallel to the tubes and about two inches from the outermost. A duplicate set of 54 sockets was installed above these on the opposite side of the board and they were so wired that the three lamps of any series could be all above or all below the board or one above and two below or two above and one below. This was done so that the amount of radiant energy reaching the plants from the ballast could be readily controlled.

The wiring diagram of such a panel is very simple. Three 60-watt, 115-volt incandescent-filament lamps are wired in series with one fluorescent lamp. The entire bank of 18 fluorescent lamps and 54, 60-watt, 115-volt filament lamps draws 8.3 amperes at 453 volts A.C. The total load of this panel is approximately 3420 watts at a power factor of about 91%. The load on each fluorescent lamp with the three 60-watt filament lamps is 190 watts, 124 watts are consumed by the three 60-watt filament lamps operating at about 90 volts each and about 66 watts are consumed by the slimline lamp. The filament lamps have an extremely long life since they are operating at low voltage.

The initial light intensity of a battery of 4500° white lamps as measured at the center of the panel by a Weston sunlight meter at 18 inches distance was approximately 1200 foot-candles. After 300 hours of burning the intensity decreased to 1000 fc and remained rather constant for 2000 hours. After this time the intensity gradually dropped to around 700 fc after 3000 hours.

Experimental procedure

In each experiment soybeans were grown with three different sources of radiant energy. One source was the panel of slimline fluorescent lamps with or without the radiation from the incandescent-filament lamps, another was a carbon-arc lamp burning "Sunshine" carbons supplemented with incandescent-filament lamps (**6**), and the third was solar radiation as received in the greenhouse. The first two sources were in separate controlled-environment rooms which have previously been described (**6**). The photoperiod was 16 hours in all experiments. Such a photoperiod was provided in the greenhouse by extending the period of natural radiation with about 30 fc of light from incandescent-filament lamps. For each experiment there were 133 plants in the fluorescent-lamp room, 140 in the arc-lamp room and 50 in the greenhouse.

Cultural conditions were identical under all three sources. Three Biloxi soybean seeds were planted at a uniform depth in white quartz sand in small glazed crocks that were 4 inches in diameter and 8 inches in height and had a side drain at the base. The pots were irrigated daily with a complete nutrient solution (**6**). Six or seven days after germination the seedlings were thinned to one per pot. Temperature in the two controlled-environment rooms was maintained at 70° F until the seedlings emerged from the sand. At this time the lights were started and a temperature of 80° F was maintained during the 16-hour photoperiod and 70° F during the 8-hour dark period. Temperature in the greenhouse was maintained near 70° F in the winter but was not controlled at other times.

The plants were harvested beginning at 1 P.M. on the 21st day from time of seeding. Each plant was cut at the sand level, fresh weight of individual plants was determined, and the length of each internode of each plant was measured. The dry weight of the entire lot was determined after the plant had been dried at 80° C in a forced-draft oven.

Results

The averages of the results of three separate experiments in which 4500° white fluorescent lamps were used as the only source of irradiation were compared with the averages of three other experiments in which the same fluorescent source was supplemented with the radiation from the 54, 60-watt incandescent lamps that served as ballasts (table I). The fresh and dry weight of the plants was greater and the stem length was less when the radiation from the fluorescent lamps was supplemented with that from the incandescent-filament lamps.

Plants grown with radiation from carbon-arc lamps, supplemented with incandescent lamps were always shorter and heavier than those grown with radiation from fluorescent lamps. In general, the plants all had a good healthy appearing green color. The leaf area of those in the arc-lamp room was obviously greater than that of plants in the fluorescent-lamp room or in

the greenhouse but leaf areas were not actually measured. The stems of the plants in the arc-lamp room were very much sturdier than those in the fluorescent-lamp room and were of greater diameter.

Soybeans grown in the greenhouse during the fall and winter months were not so heavy as the plants grown with radiation from the fluorescent lamps and were usually much shorter. However, during the spring months the plants grown in the greenhouse were about the same quality, as judged by stem length and weight, as those grown with radiation from the fluorescent lamps.

TABLE I

THE AVERAGE WEIGHT AND STEM LENGTH OF BILOXI SOYBEAN GROWN FOR THREE WEEKS ON A 16-HOUR PHOTOPERIOD WITH FOUR DIFFERENT SOURCES OF RADIATION. THE RESULTS ARE THE AVERAGES OF THREE SEPARATE EXPERIMENTS FOR EACH SOURCE.

SOURCE OF RADIATION	FRESH WT. PER PLANT	DRY WT. PER PLANT	LENGTH OF STEM IN MM.					
			TOTAL	HYPOCOTYL	FIRST INTERNODE	SECOND INTERNODE	THIRD INTERNODE	FOURTH NODE TO TERMINAL
	gms.	*gms.*						
8′-Slimline 4500° White Fluorescent	8.77	1.03	384.8	68.3	92.9	37.6	63.4	122.6
8′-Slimline 4500° White Fluorescent plus Incandescent	10.22	1.26	334.5	56.3	85.5	57.7	78.1	56.9
Arc-Lamp plus Incandescent	11.87	1.66	240.5	63.0	77.0	39.4	37.9	23.2
Greenhouse								
Winter	5.16	0.48	291.9	79.7	102.4	60.6	30.7	18.5
Spring	8.70	1.16	317.0	62.7	90.0	45.1	59.3	59.9

Discussion

A limiting factor in most controlled-environment rooms is the radiant energy. Therefore, every effort is usually made to increase this factor as much as possible within the limits of the source. When the panel of slimline lamps described was first constructed the only induction ballasts available were 100 and 200 milliamperes and with these the fluorescent lamps gave lower radiant emittance than with the incandescent lamp ballast. However, 300-milliampere ballasts are now available and the radiant emittance of the 8-foot slimline lamp is approximately the same when operated on either this induction ballast or the resistance ballast. Since the circuit described requires 460 volts and generates a great amount of heat its usefulness will be definitely limited. It is probable that a panel using 300 milliamperes or higher inductive ballasts will be more adaptable than the circuit described.

Although the plants from the arc-lamp room were of considerably better quality than those from the fluorescent-lamp room, this does not neces-

sarily mean that the spectral quality of radiation from the fluorescent lamps was less suited to the requirements of soybeans than the radiation from the arc-lamp. This difference in plant quality could well have resulted from the difference in energy alone. Intensity at the leaf surface in the arc-lamp room was about 2000 fc while in the fluorescent room it was never greater than 1200 fc exclusive of the light from the filament lamps, which was about 130 fc.

Soybeans grown with radiation from 4500° white fluorescent lamps, supplemented with the radiation from the incandescent-filament lamp ballasts, were heavier and the stem lengths shorter than those grown without the supplemental radiation. These beneficial effects on growth may have been due to an improved spectral distribution of the radiation or simply to the additional energy that was provided. However, since red light has been found to be so effective in many regulatory reactions (**4, 5**) it is probable that the radiation from the supplemental incandescent-filament lamps was most effective in controlling reactions of this type. Therefore, even though the fluorescent lamps are energized with inductive ballasts it may prove desirable to supplement their radiation with a small amount of radiation from incandescent lamps operating on a separate circuit. Experiments are in progress to determine how much supplementary incandescent radiation is necessary to produce maximum growth of soybean.

Summary

1. A circuit for operating 8-foot slimline fluorescent lamps with incandescent-filament lamps as resistance ballast is described.

2. The growth of soybeans as measured by weight and length of stems was compared when grown with four different sources of radiation.

3. Plants grown with the radiation from an arc lamp supplemented with incandescent-filament lamps were heavier and the stem lengths were shorter than those grown with any of the other sources tested.

4. The radiation from 4500° white 8-foot slimline fluorescent lamps produced heavier plants with shorter stems when supplemented with the radiation from incandescent-filament lamps. These plants were superior to those grown in the greenhouse during the winter months and were comparable to those grown with natural radiation in the spring.

The authors wish to express their thanks to Mr. Lowell E. Campbell of the Division of Farm Electrification for the electrical measurements of the circuit.

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RESERVE CARBOHYDRATES IN ORCHARD GRASS CLIPPED PERIODICALLY[1]

V. G. Sprague and J. T. Sullivan

(WITH FOUR FIGURES)

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Most perennial plants undergo annual cycles of activity but forage plants, as a result of periodic defoliation, are compelled to undergo several cycles in one year. Much experimental work has been conducted to determine the yield, chemical composition, and in some cases the nutritive value of top growth removed by successive cuttings or grazings. The growth processes of the plant during these periods have received only limited attention so that changes that occur between two successive cuttings in those parts of the plant other than the edible tops have been deduced from occasional and usually not continuous observations. As part of a series of experiments concerned primarily with the reserve carbohydrates of underground parts, some of the changes that took place in the stubble and roots of perennial ryegrass when the plants were defoliated have been described (**4**). In the present paper chemical analyses of orchard grass subjected to three successive cuttings are reported. The objectives of this work were twofold: first, to follow the fluctuations of carbohydrate reserves of the stubble and roots when orchard grass is clipped periodically and maintained in a vegetative condition, and second, to note the effects of nitrogen fertilization upon these changes. The results indicate that a cycle of compositional changes recurs after each cutting.

Experimental procedure

The plant material used in this experiment was obtained by vegetative propagation from a vigorous clone of orchard grass, *Dactylis glomerata* L. Established plants of this clone were broken up to provide numerous vegetative tillers. All roots and old leaves were removed and the tillers were placed in tap water for several days. When new roots appeared, three tillers were planted in each of a number of 1-gallon glazed crocks. The potting soil consisted of two parts of Hagerstown silty clay loam and one part of river bottom sand. Adequate moisture was applied and maintained throughout the experiment. The crocks were arranged on four parallel benches in one section of a greenhouse. The temperature of the greenhouse was maintained at about 70° F and the day length was extended to 16 hours by supplementary Mazda light. The few heads that formed during the two-month period of establishment were removed.

On January 4, 1944, all plants were clipped at 1.5 inches above ground level, the required number of plants were removed for chemical analysis,

[1] Contribution No. 94 of the U. S. Regional Pasture Research Laboratory.

and the remainder were fertilized. The fertilizer applications, made in water solution, consisted of the addition of monopotassium phosphate, at a rate of 200 pounds per acre, to all pots, and of urea, at a rate of 30 pounds of nitrogen per acre to one-half of the pots, the "low nitrogen" series, and at a rate of 90 pounds per acre to the other half, the "high nitrogen" series. Thirty-five days later the plants remaining were cut again at 1.5 inches above ground level and urea was again applied, at a rate of 40 pounds N per acre to the low nitrogen series and at 150 pounds N per acre to the high nitrogen. A third cutting was made 35 days after the second and a third addition of nitrogen at 50 pounds N per acre to the low nitrogen series and at 150 pounds N to the high nitrogen.

Plants were removed for analysis at 3, 7, 14, 21, and 35 days after each cutting. In these samplings the plants from three pots, selected at random from each series and each replication were combined, and after washing off the soil, were separated into tops, stubble, and roots. Since orchard grass grown continuously under a long day does not flower, the tops were composed entirely of leaf growth above the 1.5 inch level. The stubble included the remainder of the plant except for the roots and consisted almost entirely of leaf tissue. This was largely leaf sheaths since the blades were removed with the tops, and since the stems in vegetative tillers of this grass species are very short.

The three plant parts were preserved separately in boiling alcohol and later, after coarse grinding, were extracted completely with 80% alcohol. An aliquot of each alcoholic extract was used to determine total solids. The sum of the weights of the total solids of an extract and of the corresponding alcohol-insoluble residue gave the total dry weight of that sample, which was also the dry weight yield of that plant part at the time of sampling. Other aliquots of the extracts were examined for reducing sugars, total sugars, total soluble nitrogen, and qualitatively for nitrates. Reducing power was determined by a method essentially that of PHILLIPS (**2**) except that the reduced copper was removed by centrifuging and determined by titrating with ceric sulphate. Total sugars were determined after hydrolyzing the sucrose with dilute HCl at 70° C. Sucrose was calculated as the difference between total and reducing sugars and both are reported as sucrose. Portions of the alcohol-insoluble residue (after fine grinding) were used for fructosan and for total nitrogen determinations. Fructosan was determined by heating a 1-gram portion with 0.25% oxalic acid for one hour at about 80° C and examining the extract for fructose by the method of PHILLIPS and SMITH (**3**), a modification of that of VAN DER PLANK (**5**). The fructose was considered to have been derived from fructosan and is so reported, but expressed as fructose. Total nitrogen is the sum of the soluble nitrogen (total nitrogen of the alcohol extract) and of the insoluble nitrogen. All constituents are reported in percentages of the dry weight and soluble nitrogen is also reported in percentage of total nitrogen.

Experimental results

The distribution of soluble carbohydrates in orchard grass plants is not essentially different from that previously reported in ryegrass (**4**). A preliminary analysis of orchard grass which had been uncut for several months in the greenhouse is listed in table I. Leaf blades were higher than the stubble in sucrose, but lower in fructosan. In leaves and stubble the lower zones of each were richer than the upper zones in fructosan, the most abundant constituent, and in total soluble carbohydrates. Clipping (or grazing) would therefore tend to remove an increasing proportion of reserve substances as the clipping was made closer to the ground level. Roots are relatively high in sucrose but are lower than the tops in percentages of total carbohydrates; however, because of their considerable size—over 40%

TABLE I

PRELIMINARY ANALYSIS OF ORCHARD GRASS TO SHOW DISTRIBUTION OF CARBOHYDRATES IN DIFFERENT PARTS OF THE PLANT.

PART OF PLANT	WEIGHT OF PLANT PART (IN PERCENTAGE OF WEIGHT OF ENTIRE PLANT)	CARBOHYDRATES (IN PERCENTAGE OF DRY WEIGHT)		
		REDUCING SUGARS	SUCROSE	FRUCTOSAN
Upper ½ of leaf blades	14.0	1.4	8.4	7.6
Lower ½ of leaf blades	12.1	1.2	5.8	22.0
Upper ½ of stubble*	9.4	1.9	3.6	23.7
Lower ½ of stubble	23.6	0.7	2.6	36.2
Roots	40.9	1.2	8.9	8.2

* Stubble includes all vegetative plant parts other than leaf blades and roots.

of the whole plant in the present case—they contain a large part of the available reserves. This distribution of reserves seems to be typical of all grasses of similar anatomical structure but may be modified considerably by cultural practices and environmental factors.

The changes that took place in orchard grass during the three recovery periods between cuttings are presented in a series of graphs, figures 1–4. For economy of space only the mean dry weight and mean analytical results of the two replicates were used in making the graphs.

In figure 1 are presented four graphs illustrating the dry weights of the entire plant and severally of the tops, stubble, and roots. The weight of the entire plant (fig. 1A) gained almost steadily with time during each intercutting period, except that a slight loss may have occurred during the first few days in the high nitrogen series, as a result of increased respiration following the clipping stimulus. This gain in whole plant weight was primarily owing to new top growth (fig. 1B) and to a less extent to stubble growth (fig. 1C) and root growth (fig. 1D). (Note that figs. 1B, 1C and 1D are on a larger scale than fig. 1A). The stubble and roots continued to grow, both having approximately doubled in their dry weight by the end

of the 15-week period. Part of the stubble increase was caused by the formation of new leaf sheaths and blades below the cutting plane. It is to be noted that increases in stubble weight (fig. 1C) and also root weight (fig. 1D) often occurred toward the end of each intercutting period, and not so much during the early parts. Some losses in weight are noticeable in the roots during the first few days after cutting and may have been caused by translocation or the disappearance of certain constituents.

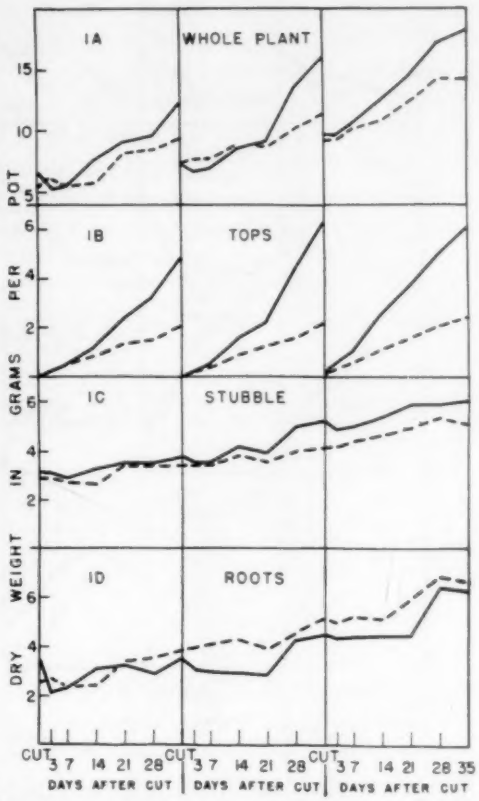

FIG. 1. Dry weight changes of the whole plant, tops, stubble, and roots through three intercutting periods. Under high nitrogen (solid lines) and low nitrogen (broken lines) nutrition.

High nitrogen fertilization, as compared with low nitrogen, gave a larger yield of the whole plant, but the greater part of the increase was in the tops, with a smaller part in the stubble. In the roots the reverse was true, the roots being heavier under the lower nitrogen fertilization. Published reports concerning the effects of fertilizers upon the growth of roots are sometimes contradictory but the depressing effect upon root weight by nitrogenous fertilizers has been noted in a number of instances (**6**). In other cases root yields were not suppressed by nitrogenous fertilizers, but the root-top ratio was lowered because the roots were not stimulated by nitro-

gen to the same extent as were the tops. In this experiment the weights of the low nitrogen roots were significantly greater than the high nitrogen for a period of five weeks after each application but there is a suggestion that the superiority of the low nitrogen roots became less with the passage of time and might not have continued if the intercutting periods had been lengthened. Quoting from WEINMANN (6), "an abundance of nitrogen

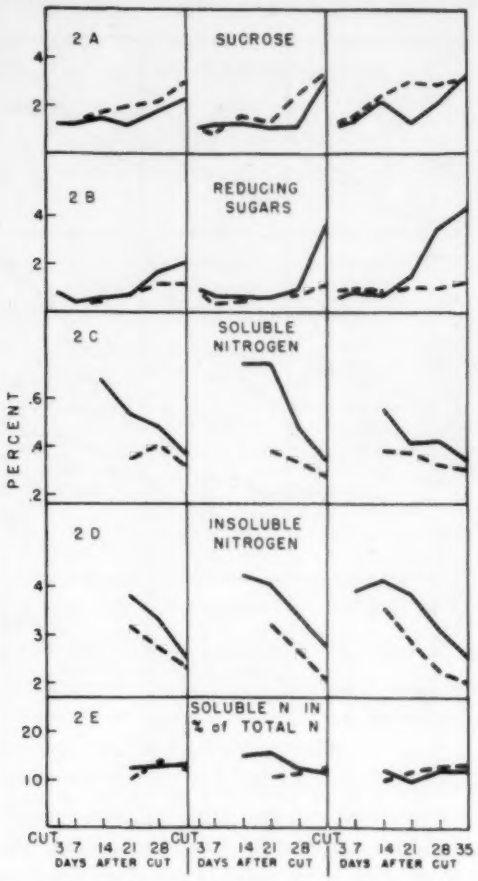

FIG. 2. Carbohydrate and nitrogen contents of the tops (above 1.5 inches) during three intercutting periods. Under high nitrogen (solid lines) and low nitrogen (broken lines) nutrition.

may, at certain stages of the plant's life cycle or under conditions of severe defoliation, lead to an increased utilization of carbohydrate reserves and even to a reduction in root weight. . . . The increase in total leaf area . . . usually associated with improved soil fertility, may ultimately result in an increased accumulation of reserves and higher root weights."

The composition of the tops is presented in figure 2. While the carbohydrates of the tops may not function as reserves, their analysis aids in the

interpretation of the composition of the stubble, since the stubble includes some new leaf growth. The amounts of new top growth produced during the first few weeks after each clipping were very limited and, as a result, nitrogen determinations had to be omitted in some of the smaller samples and fructosan was determined in only the largest samples.

The sugar content of leaves increased as they became older, slowly at first but more rapidly at two to four weeks after cutting when the leaves were more mature. Sucrose was the dominant sugar up to the fourth week, but beyond that a different relationship occurred. The reducing sugars of the high nitrogen series increased rapidly and exceeded markedly those of the low nitrogen series and also exceeded sucrose. This differential action of the level of nitrogen toward the two types of sugars is of particular interest in explaining contradictory conclusions about the effect of the fertility level on the chemical composition. If plants were collected for analysis only once and that at a time soon after clipping, no significant effect

TABLE II

FRUCTOSAN CONTENT OF ORCHARD GRASS TOPS, IN PERCENTAGE OF DRY WEIGHT.

TIME OF SAMPLING	LOW N NUTRIENT		HIGH N NUTRIENT	
	Bench 1	Bench 3	Bench 2	Bench 4
35 days after first clipping	1.98	1.67	Trace	0.74
28 days after second clipping	*	*	Trace	Trace
35 days after second clipping	6.78	3.61	1.38	0.75
28 days after third clipping	1.76	Trace	None	None
35 days after third clipping	1.90	0.76	0.95	Trace

* Not determined.

of nitrogen on the sugar content would be noted. If collected at a somewhat later date the effect of nitrogen in depressing sucrose would be evident, and at a still later date its effect in increasing reducing sugars without affecting sucrose. If total sugars only were determined on such collections, no differential effect attributable to nitrogen would be found for the first two weeks after clipping; for the next week or two nitrogen would depress total sugars and for the remaining time it would markedly increase total sugars. KUHN and KEMP (1) analyzed red fescue at the full bloom stage. It was reported that total sugars in plants fertilized with a complete nutrient exceeded those in unfertilized plants but whether this excess was caused by increased reducing sugars or by other sugars was not stated.

The fructosan content of the tops is presented in table II. Fructosan was not found in this part until late in the intercutting period and was slower to appear in the high nitrogen series where growth was more rapid. The lack of close agreement between replications characterizing the fructosan content of tops may perhaps be explained by the sudden appearance of fructosan at a certain stage of plant maturity, its very rapid accumulation

from that time on, and by slight variations in the plane of clipping when a steep gradient of fructosan occurred.

Both soluble nitrogen (fig. 2C) and insoluble nitrogen (fig. 2D) decreased at a uniform rate as the leaves aged. The high nitrogen series contained more soluble and insoluble nitrogen than did the low nitrogen series and in approximately the same proportions (fig. 2E). Nitrates were pres-

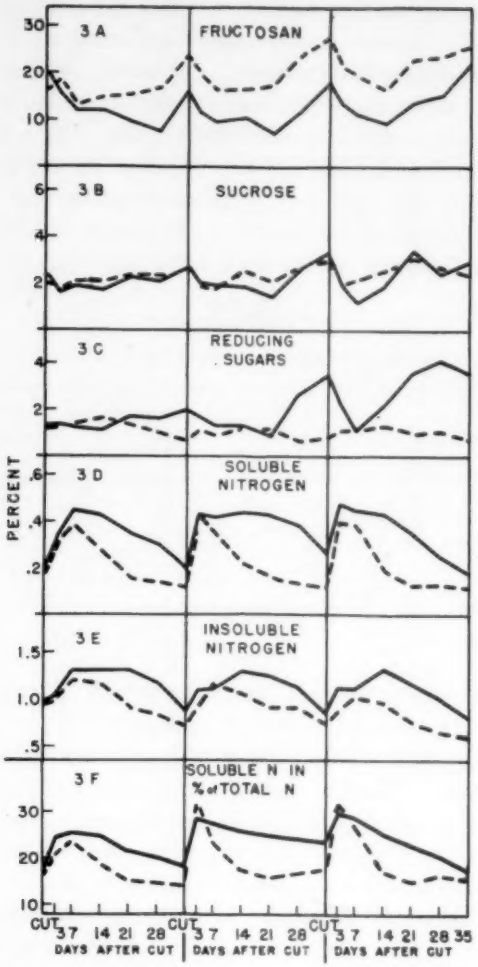

FIG. 3. Carbohydrate and nitrogen contents of the stubble. Under high nitrogen (solid lines) and low nitrogen (broken lines) nutrition.

ent in the tops after each addition of urea to the soil; they persisted for two weeks in the low nitrogen series and for two to five weeks in the high nitrogen series.

In the stubble (fig. 3), fructosan (fig. 3A) was the most dominant of the soluble carbohydrates. Beginning at a high concentration of nearly 20% of the dry weight it decreased rapidly after clipping and reached a

low point, after which it increased. This fluctuation conforms to the U-shaped curve referred to earlier (**4**) whereby fructosan in the stubble was interpreted as acting as a reserve, being withdrawn to furnish raw material for the formation of new tissue and being replaced when that new tissue was able to manufacture carbohydrate in excess of its own immediate needs. Sucrose (fig. 3B), though smaller in amount, (note that figure 3B is on a larger scale than figure 3A), is in a similar category. Some of these carbohydrates, perhaps after hydrolysis, were consumed in respiration since there was a loss in total plant dry weight after clipping (not counting the removed tops).

Reducing sugars have not been considered to be important as reserve substances in grasses. Nevertheless in these experiments they do appear to be in this category at certain times. The U-curve is not noticeable after the first clipping, but is present after the next two clippings in the high nitrogen series. Since the high nitrogen series were the highest in percentage reducing sugar, it appears that utilization of reducing sugars took place only when reducing sugars were high, for example, over 1.7%, but not when at lower concentrations. With sucrose also, utilization was greater when it was present in larger amounts. High nitrogen, by stimulating rapid growth, tended to hold fructosan at a lower level at all times, to maintain sucrose at a slightly lower level during the early parts of the intercutting periods, and to increase reducing sugars, especially during the later parts of the same periods.

Both the soluble nitrogen (fig. 3D) and the insoluble nitrogen (fig. 3E) of the stubble increased sharply after clipping and then decreased gradually. The high nitrogen series reached higher maxima than the low nitrogen and were slower in falling from them. There is a suggestion that, if the recovery periods had been long enough, the high and low nitrogen series may have become alike in per cent. nitrogen, the higher application of nitrogen showing its effect largely in a higher dry matter yield. Nitrates persisted in the stubble for some time after each addition of urea, for three to 14 days in the low nitrogen plants and for four to five weeks in the high nitrogen.

In the roots (fig. 4), the fall and rise of the various constituents resembles in some degree that which took place in the stubble. The evidence of utilization of fructosan after clipping is not as marked; but sucrose on the other hand was higher in the roots than in the stubble and showed greater utilization and restorage. Reducing sugars, as also in the stubble, showed utilization only when they were relatively high. The effects of nitrogen level are similar to those in the stubble; high nitrogen as compared with low nitrogen lowered the fructosan content and in most cases lowered sucrose but it had decidedly increased reducing sugars by the end of the 5-week period. The increased reducing sugars were not derived from either sucrose or fructosan since no loss of either of these latter occurred

during that period of time anywhere in the plant. Soluble nitrogen of the roots increased sharply after the cutting of the tops and the application of urea. There is evidence from other work to indicate that either one of these processes and not just the addition of fertilizer brings about a rise in soluble nitrogen. Nitrates were not usually present in the low nitrogen

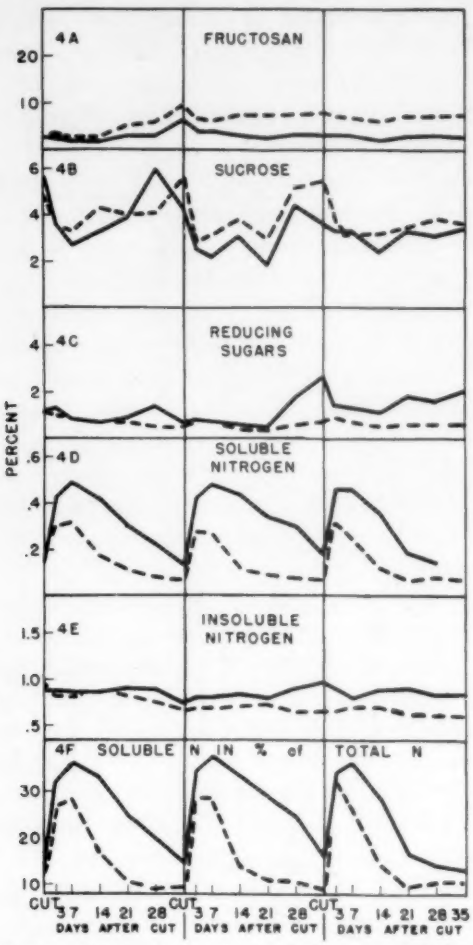

FIG. 4. Carbohydrate and nitrogen contents of the roots. Under high nitrogen (solid lines) and low nitrogen (broken lines) nutrition.

roots, having disappeared a week after the plants were first cut, but appeared and persisted for about four weeks after each addition of urea to the high nitrogen series.

The three carbohydrate fractions, reducing sugar, sucrose, and fructosan, which appear to be possible reserve substances, have been found to vary throughout the plant and at different fertility levels when expressed on a percentage basis. When based on the absolute amounts per plant rather than as percentages some different relationships might hold. The

two methods of expression are compared in table III for three dates, the dates chosen being one at the end of each of the three intercutting periods. The data indicate that the fructosan content of the stubble produced under the moderate nitrogen fertilizer (low N) exceeded that grown under high nitrogen, not only on a percentage basis but also in absolute amounts. While the per cent. sucrose was about the same in the two different groups of stubble a greater absolute amount of sucrose was present in the high N stubble than that grown under the low N conditions because of the greater yield of the high nitrogen plants. Also, on the absolute basis, the effect of high nitrogen in increasing reducing sugars in the above-ground parts

TABLE III

A COMPARISON OF THE PERCENTAGE BASIS WITH THE ABSOLUTE BASIS OF EXPRESSION OF RESERVE CARBOHYDRATES, MADE AT THREE DATES, VIZ. AT 35 DAYS AFTER EACH OF THE THREE CUTTINGS*

Intercutting period (sample taken on last day of each)	Stubble				Roots			
	Low N		High N		Low N		High N	
	%	mg. per pot	%	mg. per pot	%	mg. per pot	%	mg. per pot
				Fructosan				
first	24.1	838	16.3	662	9.8	376	6.3	228
second	27.6	1140	17.8	934	8.2	421	3.2	139
third	26.2	1334	22.0	1315	7.4	491	2.8	179
				Sucrose				
first	2.58	89	2.76	105	5.60	216	4.26	150
second	3.04	125	3.26	172	5.45	276	3.58	150
third	2.50	128	2.84	169	3.52	236	3.38	212
				Reducing sugars				
first	0.76	26	2.02	77	0.50	20	0.71	25
second	0.82	34	3.56	185	0.68	35	2.66	118
third	0.74	38	3.64	219	0.67	45	2.00	125

* Each figure is the average of duplicate samples each containing the plants from three pots.

seems greater than it did on a percentage basis. Because the roots under the low nitrogen level have more dry matter and also more fructosan and sucrose, they gain added importance over the high nitrogen roots as reservoirs of these carbohydrates when under the absolute system of expression. However, the stubble, under both levels of fertility and by either method of expression, remains the chief storehouse of reserve carbohydrates.

Conclusions and summary

Orchard grass plants derived from a single clone were grown in the greenhouse under two levels of nitrogen fertility and clipped every five weeks. The tops, stubble, and roots underwent recurring and cyclic changes in dry weight and in some phases of chemical composition during three intercutting periods.

While final dry weight yields of the tops and stubble were higher under high nitrogen than under low nitrogen nutrition, root weights were greater under low nitrogen.

Total water soluble carbohydrates were higher in the stubble than in the tops and roots and this could be largely attributed to the predominance of fructosan. After each cutting of the tops, fructosan and sucrose of the stubble and roots decreased in percentage in accordance with their probable role of reserve substances. The decrease in each case seemed proportional to their concentration, greater decreases occurring, particularly in the roots, when their percentages were high. Reducing sugars decreased after cutting only when, under conditions of high nitrogen nutrition, their percentages were high. After initial drops the percentages of all soluble carbohydrates were restored before the time of the next cutting.

Under high nitrogen nutrition the percentages of fructosan and sucrose were less than under low nitrogen. The addition of nitrogen at the higher level, while not affecting the reducing sugar content immediately, caused striking increases in reducing sugars a few weeks later and then these sugars apparently acted as reserve substances.

The nitrogen was applied to the soil in the form of urea at the time of each cutting of the tops. Within three days after these additions, marked increases of soluble nitrogen occurred in both roots and stubble and of insoluble nitrogen in the stubble. In general, these values returned to their former level by the end of a 5-week recovery period.

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GREENHOUSE TESTS WITH CHEMICALS FOR SUPPRESSION OF LATERAL BRANCHING OF DECAPITATED TOBACCO PLANTS

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Development of a simple and inexpensive method of suppressing axillary branching would be a matter of practical importance to tobacco growers. This has already been pointed out in a preliminary report of this work (**10**). Suckering or the removal of branches, is commonly practiced after topping, or decapitation of the tobacco plant at the bud or flower stage. More or less frequent suckering of the plants may be required for a period of from two to eight weeks, depending on the type of tobacco. Its purpose is primarily to improve quality of the leaf, but it is also alleged to increase yields under favorable conditions. The use of chemicals to suppress lateral branching might, if effective, possess the additional advantage of favorably modifying the quality of some types of tobacco (**3, 6, 8**). Obviously, it would be of the greatest importance to make certain that no harmful effects would follow such use of a chemical in commercial practice before recommending it for this purpose.

The mechanism by which development of lateral buds is inhibited was a subject of study (**9**) even prior to the isolation of auxins a and b by KOGL (**5**), and of heteroauxin, or indoleacetic acid the growth hormones of green plants. That inhibition of axillary branching is due to formation of auxin in the terminal bud and its excessive accumulation in the lateral buds appeared probable from the results of experiments by THIMANN and SKOOG (**11**). HITCHCOCK (**4**) found that indoleacetic acid, indolepropionic acid, ethylene, and propylene each inhibited axillary branching of decapitated tobacco plants. Since discovery of the hormonelike actions of many organic laboratory chemicals (**13, 14**) studies have been intensified. Reviews of the extensive literature will be found in BOYSEN-JENSEN (**2**), WENT and THIMANN (**12**), and AVERY, JOHNSON, ADDOMS, and THOMSON (**1**). MITCHELL and MARTH (**7**) should also be consulted on the many potentialities of these compounds.

The purpose of the experiments reported here was to obtain data on the comparative effectiveness of the available chemical compounds as substitutes for manual suckering. That is to say, the degree to which development of the axillary buds can be suppressed in the absence of the terminal bud without injury to the plant. It was realized that results of greenhouse tests might not hold quantitatively in the field. Moreover quality of cured leaf could not be studied with plants grown under greenhouse conditions, since such an environment is unsatisfactory for growing commercial

tobacco. The ability to screen out compounds on the basis of their failure to inhibit branching, would, however, justify use of the greenhouse procedure.

Suppression of suckers, even if complete, has nevertheless not been used as the sole criterion of effectiveness. Observations, particularly concerning leaf distortion and other abnormal growth effects, weight of leaf yield and uniformity as between individual plants in each lot, were made and influenced the choice of treatments in succeeding tests. These and still other factors such as difficulties associated with treatment, while not discussed in detail, have influenced the planning of successive experiments and the interpretations placed on the data.

From the physiological point of view the data here included comprise tests on the relative ability of compounds to inhibit branching in the absence of the terminal bud together with the other growth effects accompanying this action.

Experimental procedures

The plants used in these experiments were the tobacco varieties Connecticut Broadleaf (*Nicotiana tabacum* L.) and Olsen 68 (*N. rustica* L.). They were handled in the customary manner and finally transplanted singly to 1-gallon glazed crocks, in which they completed their growth. Spacing of plants on the benches was on 2-foot centers. A teaspoonful of 4–8–12 fertilizer was added to the soil in each crock at intervals of six to eight days during the later stages of growth. Yellowing of the basal leaves was prevented in this way. Greenhouse temperature was maintained, so far as possible, at 70° to 80° F.

Treatment of a plant began with removal of injured bottom leaves and cutting the stem to remove terminal flowering parts and some of the youngest leaves. Some relatively small and undeveloped leaves were retained to make up the fixed total number of leaves per plant. As soon as a lot of three plants had been topped they were treated either by wetting the cut with a fixed number of drops of the chemical, or by pressing the powdered or crystalline chemical firmly into the exposed tissues. Care was exercised to obtain completeness and uniformity of spread, and closeness of contact. Sprays were used in a few instances. These were applied primarily to the stems, but some of the spray could not be prevented from reaching the leaves. Modified procedures will be described in the text in connection with the data.

Most of the compounds used were of commercial purity only. They were obtained through the courtesy of the Dow Chemical Company, the E. I. DuPont de Nemours Company, and the Sherwin-Williams Company. Their effects on growth were quite similar to those obtained with the chemically pure acids. Absence of dosage values in the tests indicates use of the dry substance as received, whereas the number following the name of a chemical indicates dosage as drops of the liquid. An assortment of com-

mercial weed eradicators containing these compounds as a main ingredient was also tested. Samples of these were obtained through the kindness of Dr. L. W. Kephart of this Bureau. The slight discrepancies in some numerical values of the tables are due to rounding out all values after finishing computations.

Experimental results

Preliminary trials with several of the chemically pure compounds having given positive results, tests were begun in May 1946 to determine in what form the compounds could best be applied and how results might best be recorded. The compounds used were γ-(indole-3)-n-butyric acid, α-naphthaleneacetic acid, 2,4-dichlorophenoxyacetic acid, p-chlorophenoxyacetic acid, dichloro-diphenyl-trichloroethane and asparagine. All excepting the last two were employed as 1% lanolin salves as well as powders. The data of these tests revealed that the variation in the total length of all suckers on a plant roughly paralleled that of their green weight. The number of suckers per plant, however, seemed to bear no relation to the total length or weight. Because weight of harvested leaf is an important consideration the yield in grams was adopted as a basis of comparison. It also seemed a more suitable measure for comparing relative growth of leaf, stalk, and branches as influenced by chemicals.

Application of the chemicals as crystals or powder to the cut seemed on the whole to be as effective in suppressing branching as application in 1% concentration in lanolin. A comparison is difficult, however, because of the insolubility of these compounds, other than asparagine, and the relatively low quantities used in the salves. Indolebutyric acid seemed somewhat more effective than the other compounds in suppressing suckers (−56% as crystals, −44% as salve). P-chlorophenoxyacetic acid as salve and asparagine as powder increased both the total and sucker growth to some extent. The increases were 26% and 34% for the former and 12% and 38% for the latter compound. Dichloro-diphenyl-trichloroethane increased total yield by 12% but did not affect sucker formation.

Effects of modifications in the method of application of chemicals did not differ greatly from those just discussed. In one method the chemicals were applied both to the cut stem and to a freshly exposed surface of a basal leaf scar. In the other method the top of the cut stem was split for about 1 inch and all exposed surfaces treated.

In the next trial (table I), two types of control were used. In one the suckers remained unharvested until the end of the period, while in the other the suckers were removed by hand as they appeared. The duplicate lots of control plants were placed at widely different locations. Manual suckering led to a 15% increase in leaf yield, and a decrease of 68% in sucker formation as compared to the unsuckered control. The total weight per plant, however, was decreased. The value for per cent. suckers (19%) in the plants suckered by hand is based on three removals of branches at an

early stage of growth during the experiment. It is obvious that removal of the axillary buds daily would have lowered this value considerably. The intervals adopted are more commensurate with field practice even though the suckers were removed more thoroughly and at an earlier stage (3 inch) than is customary.

Only methyl naphthylacetate, one drop per plant, gave a marked decrease in branching. Not only was branching entirely suppressed, but leaf yield was equal to or greater than that obtained through suckering by hand. Indolebutyric acid was less effective in preventing sucker growth, while methyl 2,4-dichlorophenoxyacetate gave results superior to the lot suckered by hand.

TABLE I

AVERAGE GREEN WEIGHTS PER THREE CONNECTICUT BROADLEAF TOBACCO PLANTS TAKEN AT END OF 35-DAY PERIOD, JANUARY 13–FEBRUARY 17, 1947, AFTER TOPPING, AND APPLYING CHEMICALS TO THE STEM WOUND

TREATMENT (SEVEN LEAVES PER PLANT)	LEAF		STALK		BRANCH	
	gms.	%	*gms.*	%	*gms.*	%
Unsuckered control, average of six plants	127 ± 13	40	53 ± 1	17	139 ± 7	44
Suckered control, average of six plants	146 ± 0	60	50 ± 6	21	46 ± 18	19
γ-(indole-3)-n-butyric acid, C.P.	162	50	52	16	112	34
α-Naphthaleneacetic acid, C.P.	144	50	51	18	95	33
2,4-Dichlorophenoxyacetic acid	134	42	61	19	121	38
Methyl naphthylacetate, 1	166	71	68	29	0	0
Methyl 2,4-dichlorophenoxyacetate	175	54	78	24	72	22

Untabulated data showed a greater yield in leaf with 2-chlorophenoxypropionic acid and with asparagine although sucker yields were no lower. The ammonium and sodium, 2,4-dichlorophenoxyacetates were ineffective as were also 2- and 4-chlorophenoxyacetic acid; 2,4,5- and 2,4,6-trichlorophenoxyacetic acid; tertiary butylphenoxypropionic acid, and isopropyl carbanilate.

Of the untabulated data in this experiment the most important was that obtained by topping in the bud stage. Results were essentially the same as with the flowering stage, but did not increase the effectiveness of treatment. Neither was there any marked improvement obtained through the use of 2% and 5% lanolin salves of these chemicals. The only item of interest with these stronger salves was the very slight improvement obtained by an additional application to a basal leaf scar.

In the next experiment (table II) manual suckering led to a 74% drop in sucker yield and a 41% increase in leaf growth. Only treatment with methyl naphthylacetate (0.8 drop) and the acid gave approximately as good results in both respects. Other compounds were also effective but to a lesser degree. These included indolebutyric acid, and the methyl, isopro-

pyl, and amyl esters of 2,4-dichlorophenoxyacetic acid. The two methyl esters of 2,4-D used were from two manufacturers.

Other data that were obtained have not been included in the above table. The following compounds were relatively ineffective: "Lorol" ester of 2,4-dichlorophenoxyacetate, 4-chlorophenoxyacetic acid, 2,4-dimethylphenoxyacetic acid, 1,3,5-triiodobenzoic acid, and 2-chloro-5-nitrobenzoic acid Spraying of stems with 20 to 50 parts per million of indolebutyric acid gave negative results. Trial of topping and treatment in the bud stage again led to no improvement in results as compared to applications in the flowering stage.

TABLE II

AVERAGE GREEN WEIGHTS PER THREE CONNECTICUT BROADLEAF TOBACCO PLANTS TAKEN AT END OF 28-DAY PERIOD, MAY 5–JUNE 2, 1947, AFTER TOPPING AND APPLYING CHEMICALS TO THE STEM WOUND

TREATMENT (TEN LEAVES PER PLANT)	LEAF		STALK		BRANCH	
	gms.	%	*gms.*	%	*gms.*	%
Unsuckered control, average of six plants	219 ± 6	41	94 ± 3	18	227 ± 27	42
Suckered control, average of six plants	308 ± 10	67	94 ± 11	21	58 ± 10	13
γ-(indole-3)-n-butyric acid, C.P.	262	59	89	20	90	21
Indolebutyric acid	289	69	79	19	51	12
α-Naphthaleneacetic acid, C.P.	287	72	92	23	20	5
Naphthylacetic acid	308	72	88	21	29	7
Methyl naphthylacetate, 0.8	289	65	106	24	47	11
2,4-Dichlorophenoxyacetic acid	267	67	90	23	44	11
Methyl 2,4-dichlorophenoxyacetate	233	65	78	22	46	13
Methyl 2,4-dichlorophenoxyacetate	250	55	108	24	95	21
Isopropyl 2,4-dichlorophenoxyacetate, 1	263	68	103	27	23	6
Amyl 2,4-dichlorophenoxyacetate, 1	271	69	94	24	30	8
2-Chlorophenoxyacetic acid	235	47	84	17	177	36
α-2-Chlorophenoxypropionic acid	257	50	106	21	152	30

A series of tests was also made with samples of commercial weed eradicators containing 2,4-dichlorophenoxyacetic acid or one of its derivatives. A 20% solution of this compound as alkanolamine salt was ineffective for inhibiting branching when used as a spray at 60 to 200 p.p.m. Suckering was held at 14% of total yield, but leaf yield was somewhat low, *i.e.*, 259 grams. The 10% solution in glycol, and the 9.6% solution in emulsified oil were relatively ineffective at one drop per plant in reducing suckers. One drop of a 40% solution as butyl esters gave fairly good results with respect to increase in leaf yield and decrease in sucker yield. The differences in results with the various weed eradicators would indicate the existence of a physiological action by the solvent in these tests.

The data of table III show an increase in leaf yield of 11% through hand suckering and a decrease in branching of 60% as compared to the un-

suckered control. Sucker suppression was equally good or better with indolebutyric acid and its methyl ester; naphthylacetic acid and its methyl ester; and with the methyl, butyl, isopropyl, and amyl esters of 2,4-dichlorophenoxyacetic acid. Mixtures of esters of these three acids completely suppressed branching for the duration of the test, but appeared to lower leaf yields somewhat as compared to the suckered control. None of the tabulated compounds increased leaf yields to any perceptible extent above that of the suckered control, but fluctuated within the same range.

TABLE III

AVERAGE GREEN WEIGHTS PER THREE CONNECTICUT BROADLEAF TOBACCO PLANTS TAKEN AT END OF 28-DAY PERIOD, DECEMBER 18, 1947–JANUARY 15, 1948, AFTER TOPPING AND APPLYING CHEMICALS TO THE STEM WOUND

TREATMENT (SEVEN LEAVES PER PLANT)	LEAF		STALK		BRANCH	
	gms.	%	*gms.*	%	*gms.*	%
Unsuckered control, average of nine plants	194 ± 6	54	57 ± 3	16	107 ± 13	30
Suckered control, average of nine plants	216 ± 7	68	59 ± 2	19	43 ± 5	13
Indolebutyric acid	205	73	63	22	14	5
Naphthylacetic acid	218	73	65	22	17	6
2,4-Dichlorophenoxyacetic acid	214	61	71	20	63	18
Methyl indolebutyrate	193	66	56	19	45	15
Methyl naphthylacetate, 1	201	75	67	25	.....	.....
Methyl 2,4-dichlorophenoxyacetate	197	71	62	22	20	7
Butyl 2,4-dichlorophenoxyacetate, 1	192	74	67	26	1	1
Isopropyl 2,4-dichlorophenoxyacetate, 1	196	77	58	23	.....	.....
Amyl 2,4-dichlorophenoxyacetate, 1	212	66	77	24	34	11
Methyl indolebutyrate + methyl naphthylacetate (1: 2), 1	203	74	70	26	.....	.....
Methyl indolebutyrate + isopropyl 2,4-dichlorophenoxyacetate (1: 2), 1	198	80	49	20	.....	.....
Methyl naphthylacetate + isopropyl 2,4-dichlorophenoxyacetate (1: 2), 1	195	77	59	23	.....	.....

Much the same results as those given for mixtures were also given by various solutions and weed eradicators which have not been included in the above table. These included a 10% solution in xylene (+"Triton"—only designation on container) of methyl indolebutyrate (four drops); and 20% solutions of methyl naphthylacetate (two drops) and methyl 2,4-dichlorophenoxyacetic acid (two drops) in the same solvent. Among weed eradicators used the 20% solutions of the above three acids in triethanolamine were employed at two drops per plant; 2,4-dichlorophenoxyacetic acid was also included as alkanolamine salt (20%, one drop), and as butyl ester (40%, one drop).

Also included in this experiment was a test in which results on sucker suppression were compared when all, or three of the seven leaves, were removed at the top or bottom of the topped plant. One drop per plant of methyl naphthylacetate was the chemical used. The values for sucker percentage yields with three top leaves removed was 32 for unsuckered, 17 for suckered, and six for the chemical. With the three bottom leaves removed the analogous values were 26, 10, and 0; and with all seven leaves removed, 25, 11, and 0, respectively. These results would indicate that the leaves do

TABLE IV

AVERAGE GREEN WEIGHTS PER THREE CONNECTICUT BROADLEAF TOBACCO PLANTS TAKEN AT END OF 28-DAY PERIOD, APRIL 12–MAY 10, 1948, AFTER TOPPING AND APPLYING CHEMICALS TO THE STEM WOUND

TREATMENT (EIGHT LEAVES PER PLANT)	LEAF		STALK		BRANCH	
	gms.	%	*gms.*	%	*gms.*	%
Unsuckered control, average of six plants	266 ± 9	44	83 ± 7	14	251 ± 5	42
Suckered control, average of six plants	313 ± 20	69	83 ± 7	18	57 ± 4	13
Indolebutyric acid	300	64	84	18	86	18
Naphthylacetic acid	301	72	75	18	44	11
2,4-Dichlorophenoxyacetic acid	298	58	86	17	131	26
Methyl indolebutyrate	309	53	72	12	206	35
Methyl naphthylacetate, 1	303	73	96	23	16	4
Methyl 2,4-dichlorophenoxyacetate	213	73	51	17	29	10
Butyl 2,4-dichlorophenoxyacetate, 1	282	65	72	17	78	18
Isopropyl 2,4-dichlorophenoxyacetate, 1	275	64	77	18	80	19
Amyl 2,4-dichlorophenoxyacetate, 1	278	74	71	19	25	7
Butyl naphthylacetate, 1	312	65	127	27	38	8
Methyl indolebutyrate + methyl naphthylacetate (1: 2), 1	325	69	104	22	40	8
Methyl indolebutyrate + isopropyl 2,4-dichlorophenoxyacetate (1: 2), 2	220	81	50	18	1	....

not greatly influence the translocation of this compound through the stem when it has been applied to the cut end.

A portion of the results obtained in the succeeding experiment have been tabulated in table IV. Weight of leaf increased 18% and sucker formation decreased 77% through manual suckering. Almost equally good results were given by naphthylacetic acid, and its methyl and butyl esters. Methyl indolebutyrate dissolved in double the weight of methyl naphthylacetate was similarly effective.

The results of treatments with solutions are not tabulated but were equally good in two instances, namely one containing 20% indolebutyric acid in triethanolamine (two drops) and 20% naphthylacetic acid in triethanolamine. Though two drops of the 20% solution of 2,4-dichlorophe-

noxyacetic acid was effective in decreasing sucker formation by 94% in comparison with the unsuckered control, the yield of leaf was lower than the suckered control by 10%.

Two experiments on suppression of suckers were carried out with rustica tobacco. The results are not tabulated since they are much the same though not identical with those previously obtained with Connecticut Broadleaf. Leaf yields increased in the plants suckered by hand about 9% in one case and 3% in the other. Sucker formation was simultaneously decreased by 69% and 67%, respectively. Chemical treatment with methyl naphthylacetate was even more effective than manual suckering in preventing branching in these experiments. While two drops of this compound were too toxic and depressed leaf yields, one drop per plant gave a 9% increase in yield and eliminated 80% of branch growth. The methyl ester of indolebutyric acid appeared to be less effective than the free acid, though this was not the case with plants of Connecticut Broadleaf.

Triethanolamine solutions of these three acids (indolebutyric, napthylacetic, 2,4-dichlorophenoxyacetic) were more effective in depressing growth of rustica suckers than were solutions of the methyl esters in a mixture of xylene and "triton." (Only designation on container.) Sucker growth did not exceed 2, 13, and 4% of the total weight of the plants. The analogous figures for the methyl esters were 17, 17, and 17%. Leaf growth in all cases approximated or was slightly less than that of the suckered control.

Growth abnormalities caused by chemical treatment

No attempt has been made to present the data accumulated on the abnormalities in growth encountered in these experiments because of the limitations of space. The effects include callusing of the stem tissue exposed by topping, formation of root initials near the treated cut and sometimes on the petioles of upper leaves, longitudinal and transverse splitting of the stems and leaf petioles, leaf epinasty, and stem curl or kill. Sucker leaves became deformed or strapped in many instances.

In general 2,4-dichlorophenoxyacetic acid and its derivatives were most harmful in this respect, and indolebutyric acid and its derivatives least harmful. Extent of abnormality however was not wholly dependent on composition of the compound, but might be altered by differences in concentration and even the type of solvent. Extreme abnormalities were usually reflected in diminished leaf yields.

The growth abnormalities observed have however influenced the choice of compounds and the concentrations used in later experiments. The reason for this was the objective sought, namely the demonstration that chemicals could be employed to alter the proportionate growth in plant organs with a minimum of abnormalities. This meant maximum inhibition of branching with minimum inhibition of leaf growth and absence of leaf distortions. Complete suppression of branching is considered possible with

any of the effective compounds employed in concentrations and solvents capable of causing intensive and general toxicity.

Discussion of results

The data show that removal of branches at an early stage of development from decapitated tobacco plants in the greenhouse results in an increased weight of the leaves that are retained. Similar and sometimes apparently greater increases in leaf weight and suppression of branches took place with plants that had received chemical applications. In manual suckering increase in leaf yield was assumed to be due to elimination of plant organs competing for the supply of available nutrients in the plant. Chemical suckering therefore is assumed to act in a similar manner by hindering branching. Further experimentation will be necessary to decide whether increased leaf growth in both cases is equal to the degree of branch inhibition, or whether a stimulative effect is also exercised by these compounds. Two of the chemicals investigated, asparagine and p-chlorophenoxyacetic acid, apparently were capable of increasing yields of both leaves and branches simultaneously.

With the Connecticut variety the best combined results in leaf yield and branch inhibition were obtained with indolebutyric acid and its methyl ester, if absence of growth abnormalities is desired. Naphthylacetic acid, 2,4-dichlorophenoxyacetic acid, and certain of their derivatives appeared equally or more effective at times. A greater degree of abnormality was usually associated with the latter compounds, particularly 2,4-dichlorophenoxyacetic acid.

Whether chemical suppression of branching can be put into use in the production of tobacco will have to be decided on the basis of field tests. Greenhouse experiments indicate that the effects of chemical treatment are temporary and that branching of plants so treated is resumed after a period. Intercomparisons of experiments have disclosed that suppression of branching was usually less complete with plants grown in the spring than with the smaller plants grown in winter. The compounds tested, therefore, would probably be less effective with plants grown in the field under higher illuminations and less moisture.

Summary

Greenhouse plants of Connecticut Broadleaf tobacco (*Nicotiana tabacum* L.), Robinson strain, and rustica tobacco (*N. rustica* L.), Olson 68, were decapitated and chemicals were applied to the cut as powder or liquid. Among the compounds included in the tests were indolebutyric acid, naphthylacetic acid, 2,4-dichlorophenoxyacetic acid and their derivatives. Branching of the plants could be completely suppressed for a period of 28 days with certain of the compounds. Chemical suppression of branching paralleled manual removal of branches in an early stage of development

in bringing about increased weight of the leaves on the plant. Naphthylacetic acid, or derivatives, was usually most effective and 2,4-dichlorophenoxyacetic acid, or derivatives, was usually least effective. Abnormalities in growth accompanying suppression of branching were least with indolebutyric acid, or derivatives, and usually greater with 2,4-dichlorophenoxyacetic acid or derivatives. Dilute solutions of 5% to 20% seemed to be as effective as the pure compounds in some instances because of effects produced by the solvents. Absence of leaves did not interfere with chemical suppression of branching. Spraying of the plant stalks was relatively ineffective.

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HISTOLOGICAL STRUCTURE OF POTATO STEMS AND LEAVES AS INFLUENCED BY MANGANESE TOXICITY[1]

B. ESTHER STRUCKMEYER AND K. C. BERGER

(WITH FOURTEEN FIGURES)

Received August 3, 1949

Stem streak necrosis of potato has been described by BERGER and GERLOFF (**1, 2**). They found stem streak necrosis common on potatoes in northern Wisconsin grown on an acid soil. Where this disorder was prevalent the amount of manganese in the soil solution was high due to the strong soil acidity. They reported that symptoms of stem streak necrosis were first apparent on the lower part of the plants at the base of the petioles. Narrow brown streaks were formed on the lower part of the stems and petioles. As necrosis became more severe the leaves showed chlorosis between the veins and frequently brown necrotic areas appeared near the midrib of the leaflets. The streaking of the stem and defoliation of leaves progressed upward and as the stem-tip was affected, the plant died prematurely, reducing the tuber yields.

KELLEY (**4**) described the toxic effects due to excess manganese on the leaves of pineapple. In advanced stages of chlorosis of the leaves, the protoplasmic contents of the cells shriveled into a formless mass giving the appearance of partial decomposition. The chloroplasts of the palisade layer faded and disappeared and with it the starch content decreased. An accumulation of calcium oxalate crystals followed.

ELTINGE (**3**) reported on the histology of manganese deficient tomato plants. One of the first symptoms of internal manganese deficiency was injury to the chloroplasts and a decrease in size and number of starch grains in the leaves. The stems were smaller in diameter and produced a narrower band of xylem. An accumulation of calcium oxalate crystals was observed in the parenchyma cells of the stem as well as in the leaf.

Since the external symptoms of manganese toxicity have been fully described it was the purpose of this investigation to examine the internal effects of this condition on potato plants.

Materials and methods

Chippewa potatoes were sprouted in quartz sand and ten days later transferred to two gallon glazed jars containing complete nutrient solutions. The plants were placed in a wire screen which fits over the gallon jar and the roots allowed to dip into the solution. Manganese in the form of manganese chloride was added to the solution at the rates of 0.5, 5.5, 50.5, and 200.5 p.p.m. respectively. Iron was added in the form of ferric citrate at

[1] Published with permission of the Director of the Agricultural Experiment Station.

the rate of 15 p.p.m. Air was bubbled continuously through the cultures which were changed weekly in order to maintain the proper concentration of nutrients.

When the toxicity symptoms became severe (45 days) the stems and leaves were sampled for histological examination. Formalin-aceto-alcohol was used as the fixative. After dehydration with n-butyl alcohol, the samples were infiltrated with paraffin and after embedding were cut on a sliding microtome 12 microns in thickness. The sections were stained with iron-alum hematoxylin and safranin.

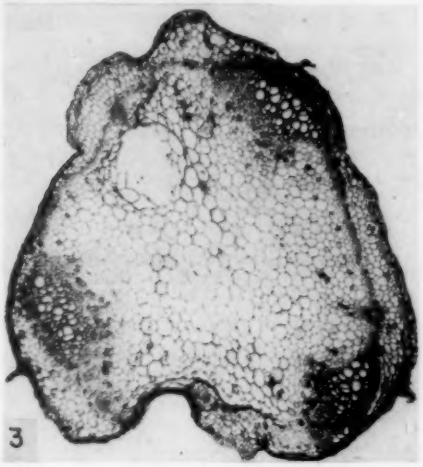
Results

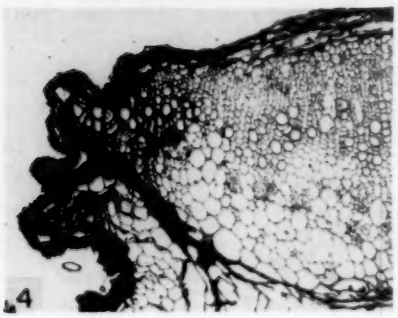
The plants grown with 200.5 p.p.m. of manganese were severely stunted. The lower leaves were brittle and dry; the upper leaves were chlorotic. Continuous brown streaks were evident along the stems and petioles (fig. 1D). The first indication of internal abnormality was the collapse of cells of the epidermal and cortical region. These collapsed regions corresponded to the brown streaks on the outer part of the stem. The internodes closer to the stem-tip showed less severe necrosis than did the lower internodes.

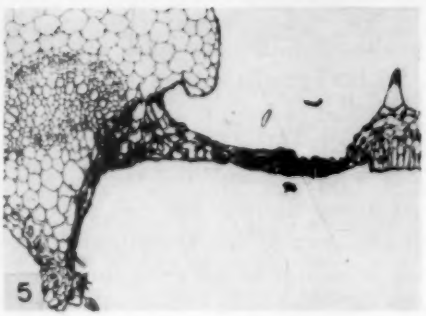
The parenchyma cells seemed to be affected by stem streak first, for it progressed from the cortex into the ray cells and then to the pith (fig. 2, 3). There was a progressive degeneration and collapse of these cells and as necrosis extended into the rays the adjacent vascular tissue became affected. There was some proliferation of cells in the phloem region, but the greatest disturbance to the vascular tissue was the displacement and injury to the cells resulting from the collapse of the surrounding tissue. Necrosis gradually extended into the pith.

In the early stages of this disorder some of the normal cells of the cortex divided to form a wound cambium which generally formed from parenchyma cells below the injured tissue. The wound cambium separated the outer injured tissue and the normal inner tissue. However, with potato stem streak necrosis the cork cells did not become suberized and therefore did not prevent the symptoms from spreading (fig. 4). The leaves had dark brown areas between the veins. Cross-sections revealed these areas to be collapsed cells. Generally all tissues of the leaf were involved so that only a dark mass of collapsed cells was apparent (fig. 5).

At a concentration of 50.5 p.p.m. the plants made considerably more growth than at 200.5 p.p.m. The leaves on the lower half of the plant were chlorotic and several of them were brittle with brown necrotic areas. Dark streaks on the stems, petioles, and leaves were conspicuous. The upper half of the plant, especially toward the tip, appeared less affected (fig. 1C). The structure of the fourth internode from the stem-tip was normal and no evidence of necrosis in any parts of the tissue could be observed (fig. 6). The ninth internode, however, showed severe symptoms of manganese toxicity. The epidermal cells and the cortical cells were again the first to col-







EXPLANATION OF PLATES

FIG. 1. Potato plants showing growth with different concentrations of manganese. A. Control, 0.5 p.p.m. manganese. B. 5.5 p.p.m. manganese. C. 50.5 p.p.m. manganese. D. 200.5 p.p.m. manganese. B, C and D showed symptoms of manganese toxicity. FIG. 2–5. Cross-sections of stems and leaf from plants grown with 200.5 p.p.m. manganese. FIG. 2. Fourth internode showing collapse and necrosis of cells. FIG. 3. Sixth internode showing necrosis. FIG. 4. Enlargement of a region in figure 2. Shows collapsed cells of epidermis, cortex and ray. Vascular tissue adjacent to ray cells also becoming necrotic. FIG. 5. Injury to leaf by manganese toxicity. Cells of leaf have collapsed and injury has extended into mid-rib.

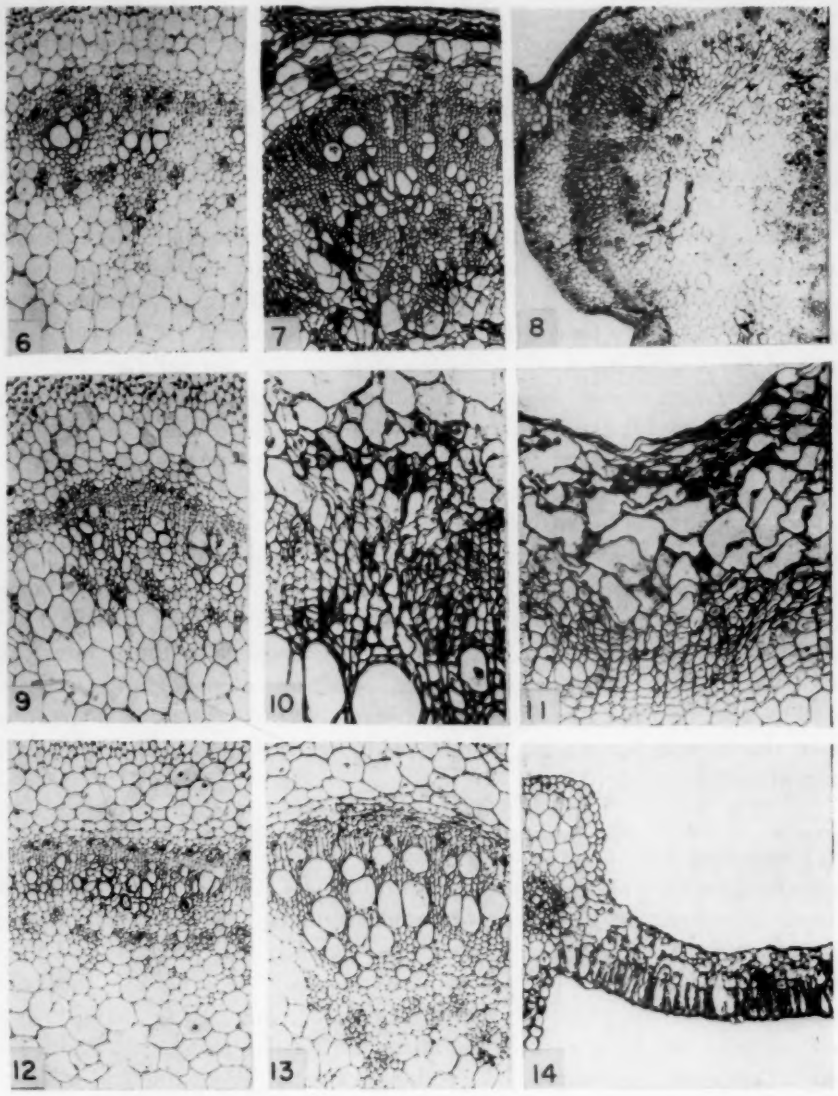


FIG. 6–8. Cross-sections of stems grown with 50.5 p.p.m. of manganese. FIG. 6. Fourth internode, cell structure normal. FIG. 7. Ninth internode, enlarged section showing necrosis in epidermis, cortex and pith. Internal phloem also injured. Wound cambium formed in cortex. FIG. 8. Ninth internode, low magnification showing necrosis in different tissues of the stem. FIG. 9–11. Cross section of stems grown with 5.5 p.p.m. manganese. FIG. 9. Fourth internode, cell structure normal. FIG. 10. Ninth internode, necrosis appearing in inner cortical cells and wound cambium forming below necrotic region. FIG. 11. Ninth internode, necrosis evident in epidermis and outer cortex. Cortical cells below necrotic region dividing to form wound cambium. FIG. 12–14. Cross-sections of stems and leaves grown with 0.5 p.p.m. manganese (control). FIG. 12. Fourth internode, cell structure normal. FIG. 13. Ninth internode, cell structure normal. FIG. 14. Leaf showing normal cell structure.

lapse and become necrotic; then xylem vessels became misshapen and misplaced as a result of collapse of the surrounding cells. Cells in the phloem region, particularly those of the internal phloem, proliferated forming a mass of disorganized tissue and within this region some cells had enlarged while others were being crushed and obliterated. Pith cells were either in stages of stretching or obliteration. Cells in the cortex had divided to form a wound cambium (fig. 7, 8).

Plants grown at a concentration of 5.5 p.p.m. manganese were greener in color and only the very lower parts of the plants showed symptoms of manganese toxicity. At this lower region there was some evidence of stem streak necrosis and a few of the lower leaves were chlorotic. The cell structure of the fourth internode from the stem-tip was normal (fig. 9). The basal internodes showed collapse and disintegration of the cells as previously described for the higher concentrations. There was a conspicuous wound cambium initiated in the cortical cells which partly separated the outer injured tissue from the normal tissue. It was not uncommon for a wound cambium to be initiated from phloem parenchyma cells, especially when necrosis had extended to the inner cells of the cortex (fig. 10, 11).

Potato plants given 0.5 p.p.m. or a normal supply of manganese, made a normal growth. They were taller than the ones previously described and of a uniform greenness (fig. 1A). Both the fourth and ninth internode from the stem-tip showed a normal stem structure (fig. 12, 13). Collapse of cells and necrosis described with higher concentrations of manganese were absent with 0.5 p.p.m. The leaves likewise showed all cells to be in normal condition (fig. 14).

Discussion

Manganese toxicity appears to affect first the outer tissues of the stem. According to KELLEY (**4**), who studied pineapple leaves, excess manganese caused a contraction of the protoplasmic contents, partial decomposition and plasmolysis of the cells. After plasmolysis the cells probably lost their rigidity and collapsed. These regions were possibly comparable to brown areas found on the chlorotic leaves of potato. Manganese toxicity may have affected the outer cells of the stem in a similar manner as it did the leaf. This would account for the collapse of cells giving the brown longitudinal streaks formed on the stem. KELLEY (**4**) and ELTINGE (**3**) reported that excess manganese and manganese deficiency had similar effects on the cell content of the leaves. The chloroplasts were the first part of the plant to be affected by either an excess or lack of this element. It was probably this condition which brought about the collapse of cells and the disorder known as stem streak necrosis.

Summary

1. Potato plants grown with 200.5 p.p.m. of manganese were stunted. The upper leaves were chlorotic and the lower ones brittle and dry. Stem streak necrosis had extended to the upper part of the stems. The cells of

the epidermis and cortex of the fourth and sixth internode had collapsed and this condition had extended into the ray cells and the pith. The vascular tissue itself was injured only slightly except for misplacement and distortion due to the collapsing tissue surrounding it.

2. At a concentration of 50.5 p.p.m. of manganese, the leaves on the lower part of the plant were chlorotic. Stem streak necrosis was apparent on the stem and petiole at this lower level of manganese. The fourth internode showed severe symptoms of manganese toxicity, such as collapse and necrosis of the parenchyma cells.

3. Plants grown at a concentration of 5.5 p.p.m. appeared normal except for the extreme lower part of the plants. The anatomical structure of the fourth internode was normal, whereas the ninth internode showed traces of stem streak necrosis in the epidermis, cortex and phloem. The wound cambium was initiated in the region of the cortex and phloem parenchyma.

4. The normal concentration of manganese for growth of potatoes was 0.5 p.p.m. The plants made normal growth, were of even greenness and were absent of stem streak necrosis. Both the fourth and ninth internodes developed a normal cell structure.

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THE MOLECULAR WEIGHT OF RUBBER IN DIFFERENT PARTS OF THE GUAYULE PLANT[1]

H. M. Benedict, P. M. Brooks, and R. F. Puckett

(WITH THREE FIGURES)

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This paper describes the routine procedure developed for the extraction, fractionation, and estimation of the molecular weight of rubber found in the guayule plant. It further presents data showing the reproducibility of the various determinations and the differences in molecular weight of the rubber in various parts of the plant such as the stems and roots.

Rubber as such is not a simple compound but consists of many compounds representing various steps in the polymerization of isoprene or a similar unit into chains of varying length resulting in molecules of different molecular weights. The factors influencing the size of the rubber hydrocarbon molecule are not too well understood. It would be of interest, therefore, to study the molecular weight of rubber produced by different parts of the plants of different ages, at various times of the year, and growing under different environmental conditions. In such studies, the rubber should be fractionated into classes of different molecular weights so that the fractional distribution as well as the mean molecular weight can be determined. Such fractionation might yield information as to the steps through which rubber of high molecular weight is built up in the plant and would certainly give a much clearer picture as to the nature of the rubber that is produced by the plants under the various conditions. For example, it can be seen that the rubber from different plants might have the same average molecular weight yet one plant might have all its rubber at that weight while the other might have half of it at a very much higher and half at a very much lower weight.

Fractionation of rubber in this manner is not new, the most recent reports being those of Skau *et al.* (**6**) primarily on goldenrod rubber and Johnson (**3**) on rubber from various sources. Skau *et al.* (**6**) and Davis and Blake (**1**) also cite several others. These methods had to be altered somewhat to obtain the information desired, hence this method employed here is given in detail.

Methods

Shrub preparation

The shrub from which the rubber is to be extracted is brought in from the field or greenhouse and immediately submerged in boiling water for 12

[1] This work was initiated by the Stanford Research Institute while under contract with the Office of Naval Research and has been continued by Division of Rubber Plant Investigations, Bureau of Plant Industry, Soils and Agricultural Engineering, Agricultural Research Administration, U.S.D.A.

minutes. (This parboiling is used in the regular milling process primarily to bring about easy defoliation. The effect, if any, of this procedure on the rubber is being investigated.) Following parboiling the leaves are shaken off, the remainder separated as desired into roots, stems, branches, etc. These various portions are cut into sections ¼- to ½-inch long, then ground in a Wiley mill using a No. 4 screen.

Extraction

After the shrub has been comminuted, 100 grams of the tissue are placed in 95% ethanol for three days (two changes of 500 ml. being used) at room temperature in the dark and shaken occasionally. This dehydrates the tissues and at the same time extracts some of the resins. The liquid is decanted off, the shrub spread out on wire trays, and the alcohol allowed to evaporate off at room temperature. The dried shrub is reground in a Wiley mill using a No. 2 screen.

The shrub is then extracted with acetone for three days in the dark at room temperature, using two changes of 500 ml of acetone. This removes 95 to 98% of the resinous materials or acetone solubles. Following extractions with the acetone, the shrub is evaporated to dryness at room temperature and 30 to 35 grams of dry shrub are placed in 250 ml. of benzene and allowed to extract for three days with occasional shaking, at room temperature in the dark. The benzene solution is decanted off, then centrifuged 60 minutes at 2000 R.P.M. The liquid is then placed in a 250 ml. volumetric flask and made up to volume. This method extracts about 80% of the rubber from the stem tissues and about 65% from the roots.

Fractionization of the rubber

As soon as the rubber is in solution an aliquot is taken for the determination of the rubber hydrocarbon content by the bromination method of Willets, Swain and Ogg (**8**), and of the total solids by evaporation. A second aliquot is taken for the determination of the mean molecular weight of the rubber in solution.

On the basis of the rubber content as determined by bromination, an amount of the rubber solution containing 1.200 grams of rubber is placed in a 400 ml. beaker and made up to 200 ml. with benzene. Methyl alcohol is then added with continuous stirring until a dense turbidity develops. The beaker is then heated to about 35° C in a water bath which results in a decreased turbidity. It is then placed in a 25° C constant temperature bath. After 15 to 20 minutes, a heavy rubber precipitate should collect in the bottom of the beaker. If such a precipitate does not come down in this time, more methyl alcohol is added, ½ to 1 ml. at a time, until it does. The amount of methyl alcohol that must be added to bring about this first precipitate varies with the concentration and molecular weight of the rubber and with the amount of solution. It is extremely important not to add too

much alcohol because, as will be shown later, it will change the results of subsequent fractionizations. Once the precipitate of rubber has settled to the bottom, the liquid remaining is carefully decanted off into another 400 ml. beaker. The precipitate is transferred to a tared weighing dish which is then dried to constant weight in a vacuum oven under nitrogen. This is the first fraction and contains the highest molecular weight rubber. Six fractions, all told, are obtained in the same manner except that after the first fraction definite amounts of methyl alcohol are added to the remaining solution of rubber. To obtain the second fraction, only 1 ml. of methyl alcohol is added in addition to that added to obtain the first fraction and the rubber is allowed one-half hour to settle out.

Continued additions and settling times are carried out as given in the following tabulation summarizing the fractionization.

Fraction	ml. of CH_3OH added in addition to amount added to obtain previous fractions	Settling Time
1	X	20 minutes
2	1	30 minutes
3	2	60 minutes
4	3	120 minutes
5	5	120 minutes
6	15	Overnight (16 hours)

This procedure allows a small portion of the rubber to remain in solution after the sixth fraction has been precipitated out. If desired, this can be recovered as a seventh and even eighth fraction. The recovery of this small quantity is very difficult and, as it would not change the picture of the fractional distribution, it is usually discarded.

In evaporating off the benzene, it has been found that the film of rubber, especially that of the first three fractions, often traps some benzene which even prolonged drying in the oven does not remove. It is therefore necessary to pull the film loose from the dish and continue the evaporation for about an hour longer.

After the benzene has been driven off, the rubber is carefully weighed and the amount recovered in each fraction compared with the total amount estimated to be present on the basis of the bromination and total solid residues. This gives the percentage of the rubber that makes up each fraction and by totaling these percentages the amount of rubber recovered can be determined.

Estimation of the molecular weight

The first step in this estimation is to determine the relative viscosity of solution of rubber in benzene as compared to pure benzene. Weighed aliquots of the dried rubber obtained in the fractionation were dissolved in known amounts of benzene, by allowing to stand in the dark for two days

at room temperature with occasional mild stirring. The viscosity of these solutions was determined at 25° + .05 C with an Ostwald viscometer similar to those used by JOHNSON (**3**). The solutions were made up to such concentrations so that the relative viscosities values ranged between 1.17 and 1.45. Experience has shown that the concentration of the benzene solutions from the various fractions should approximate the following:

first fraction	0.5 mg. per cc
second fraction	0.6 mg. per cc
third fraction	0.7 mg. per cc
fourth fraction	0.8 mg. per cc
fifth fraction	0.9 mg. per cc
sixth fraction	1.2 mg. per cc

Occasionally, with very low molecular weights, the concentration of the sixth fraction may have to be increased to 1.8 mg. per cc.

To eliminate the effect of concentration, the relative viscosities are converted to inherent viscosities according to the relation $(V) = \frac{\ln Vr}{C}$, when Vr = relative viscosity, C = concentration in grams per 100 cc, ln = natural logarithm. This value satisfactorily indicates the relative molecular weight of the rubber in solution (JOHNSON (**3**)) and in most of the literature estimations of actual molecular weights are not made. Such relative values are all that are desired for the purposes mentioned here. DEBYE (**2**) has recently discussed the viscosity method of estimating molecular weights and points out its limitations. However, approximate molecular weights may be calculated from the relative viscosity as follows:

According to DAVIS and BLAKE (**1**, p. 227), Staudinger and Heuer have demonstrated that between molecular weights of 1,000 and 10,000 of polystyrol fractions the relations $Vsp = Vr - 1 = Km\ C\ M$ holds, where

Vsp = specific viscosity
Vr = relative viscosity
Km = a molecular constant
C = concentrations grams mols per liter
M = molecular weight of the solute

$$\text{Hence} = M = \frac{Vr\text{-}1}{Km\ C}$$

$$\text{The relative viscosity} = \frac{\text{seconds for passage of solution}}{\text{seconds for passage of benzene}}$$

Km for rubber hydrocarbon (1) = .000303

C = gram per liter or mg. per cc divided by 68.11 or .01470X mg. per cc.

This relationship has been extrapolated to include molecular weights above 200,000. This is, of course, open to question but where emphasis is placed on relative rather than actual weights the error involved is not of great importance.

It is recognized that in calculating the relative viscosity the time ratio should be multiplied by the ratio of the density of solution to that of the solvent. However, in the dilute solutions used this ratio was so close to unity that its effect on the value of the relative viscosity was negligible. The constant Km also varies somewhat with the molecular weight but, since we are interested primarily in relative values only, this variation will have no effect on the interpretations of the results.

Results

To obtain some idea as to the reproducibility of the method several determinations were made on aliquots of the same solution of rubber in benzene. Table I shows the results of one such trial in which six aliquots were fractionated. As indicated by the coefficients of variability, the greatest variability occurred in the percentage of the rubber obtained in the second and third fraction. The per cent. recovered in the first fraction was the most consistent in spite of the fact that it represented more than half the rubber in the solution. The small amount of methyl alcohol that is added to precipitate the second and third fraction may, in part, account for their large variability. Slightly over 100% of the rubber estimated to be in the solution was recovered in the fractions.

The determination of the inherent viscosity of the unfractionated solution was very consistent. The greatest variation of the viscosity determinations was found in the second and third fractions as would be expected from the percentages recovered. The proportional averages are calculated by summing the products of the percentages by the viscosities and dividing by 100. These averages were slightly less than that of the unfractionated rubber but consistent between aliquots as shown by a coefficient of variability of only 4%. This emphasizes that a higher than average recovery in one fraction will be balanced by a lower than average recovery in another fraction. All the aliquots show approximately the same fractional distribution.

The results in table I give some idea as to the variability that might be expected from the fractionation procedure. The next step was to determine the variability between samples harvested from a field of guayule plants of the same age and grown under the same conditions. This would indicate how variable the guayule plants are and how much difference in molecular weight and fractional distribution would have to exist between rubber in plants treated differently to be ascribed to the treatment rather than chance variation. Table II shows the results of one trial in which the rubber from six replicates of four plants each was extracted. The plants had been grown for the same length of time in the field under identical conditions.

For the most part, the coefficients of variability for the rubber samples described in table II were greater than those in table I. This indicates that

the variability between plants exceeds that of the method and that if greater accuracy is desired more plant samples will have to be taken rather than to improve the method. The results also show that the rubber in guayule plants grown under the same conditions in the field is similar enough so that any differences in fractional distribution or molecular weight resulting from different treatments applied to the plant will not have to be

TABLE I

THE VARIATION IN PERCENTAGE AND INHERENT VISCOSITY OF RUBBER RECOVERED IN VARIOUS FRACTIONS WHEN SIX SAMPLES OF THE SAME SOLUTION WERE FRACTIONATED

SAMPLE NUMBER AND DETERMINATION	PROPORTION AND INHERENT VISCOSITY OF RUBBER FOUND IN DESIGNATED FRACTION							TOTAL	PROPORTIONAL MEAN
	UNFRACTIONATED	1	2	3	4	5	6		
1									
Percentage	100.0	48.5	32.8	7.4	5.9	4.4	5.7	104.7	
Viscosity	5.93	7.19	5.47	4.03	2.83	2.31	1.75		5.95
2									
Percentage	100.0	51.8	25.3	6.8	4.6	5.5	5.5	99.5	
Viscosity	6.12	6.92	5.24	5.20	3.19	2.49	1.84		5.65
3									
Percentage	100.0	54.2	14.2	15.6	5.1	5.2	5.6	99.9	
Viscosity	6.07	6.87	5.74	4.52	3.12	2.40	1.63		5.62
4									
Percentage	100.0	53.6	22.2	8.8	4.8	4.8	5.7	99.9	
Viscosity	6.01	6.97	5.59	3.37	3.04	2.40	1.76		5.63
5									
Percentage	100.0	59.0	19.4	10.3	6.0	4.8	5.0	104.5	
Viscosity	5.89	7.25	5.43	4.71	2.82	2.51	1.82		6.20
6									
Percentage	100.0	55.0	20.4	12.2	5.6	4.4	5.1	102.7	
Viscosity	6.08	6.67	6.19	4.23	2.87	2.28	1.74		5.80
Means									
Percentage	100.0	53.7	22.4	10.2	5.3	4.9	5.4	101.9	
Viscosity	6.02	6.98	5.61	4.34	2.98	2.40	1.76		5.81
Standard Dev.									
Percentage		3.48	6.27	3.30	.58	.44	.31		
Viscosity	.091	.214	.329	.625	.160	.092	.074		.231
Coefficient of Variability—%									
Percentage		6.5	28.0	32.4	10.9	9.0	5.7		
Viscosity	1.5	3.1	5.9	14.5	5.4	3.8	4.2		4.0

exceedingly large before they can be ascribed to treatment instead of to expected variability in the plant material.

In the course of the development of the method, certain possible causes of error or variation in the results became apparent.

The temperature at which the fractionation is carried out should be maintained as constant as possible. A decrease of a few degrees in temperature tends to increase the proportion of rubber obtained in the first fractions. In comparing the fractional distribution of the rubber in plants

that have been subjected to different treatments during their growth, it is essential that the solutions to be fractionated be made up to the same volume and concentration. Otherwise, additions of prescribed amounts of methyl alcohol in the later fractions result in different proportions of rubber precipitated.

TABLE II

THE FRACTIONAL DISTRIBUTION AND INHERENT VISCOSITY OF RUBBER IN SIX REPLICATES OF A SINGLE TREATMENT OF FIELD-GROWN GUAYULE SHRUB, FIVE YEARS OLD, FOUR PLANTS TO A REPLICATE

REPLICATE NUMBER AND DETERMINATION	PROPORTION AND INHERENT VISCOSITY OF RUBBER FOUND IN DESIGNATED FRACTION								PROPORTIONAL AVERAGE
	UNFRACTIONATED	1	2	3	4	5	6	TOTAL	
1									
Percentage	100.0	66.9	10.1	8.1	5.0	3.0	3.9	97.0	
Viscosity	6.95	8.05	5.84	4.82	3.71	3.04	1.90		6.72
2									
Percentage	100.0	60.7	18.3	9.4	1.9	3.2	2.9	96.4	
Viscosity	6.95	7.79	6.20	4.42	3.32	2.48	1.73		6.47
3									
Percentage	100.0	64.7	14.0	2.9	11.8	4.2	3.9	101.5	
Viscosity	6.90	7.66	5.81	4.44	4.40	3.03	.98		6.58
4									
Percentage	100.0	69.9	17.0	6.5	2.4	3.3	2.9	102.0	
Viscosity	6.51	7.39	5.50	4.00	3.79	2.35	1.22		6.56
5									
Percentage	100.0	59.5	22.0	6.3	4.7	3.3	4.5	100.3	
Viscosity	6.53	7.48	5.59	4.36	3.46	2.93	1.70		6.29
6									
Percentage	100.0	67.0	11.8	11.7	2.6	2.3	1.9	97.3	
Viscosity	7.13	7.48	5.59	5.10	2.90	2.18	1.50		6.42
Mean									
Percentage	100.0	64.8	15.5	7.5	4.7	3.2	3.3	99.1	
Viscosity	6.83	7.64	5.76	4.52	3.60	2.67	1.51		6.51
Standard Deviation									
Percentage		4.00	4.64	3.01	3.69	.61	.94		
Viscosity	.251	.247	.257	.505	.505	.378	.347		.148
Coefficient of Variability—%									
Percentage		6.2	29.9	40.1	78.5	19.1	28.5		
Viscosity	3.7	3.2	4.5	8.5	14.0	14.0	23.0		2.3

It is known that rubber tends to oxidize when exposed to the air, DAVIS and BLAKE (1). After the various fractions had been precipitated they had to be dried, weighed, then samples redissolved in benzene before the viscosity determinations could be made. Under normal working conditions, these procedures required as long as three days or more. It seemed that during this time considerable changes in the rubber might occur so the effects of various conditions of evaporating off the benzene, then storing the dried rubber samples, were determined. Four aliquots of a solution of rubber in benzene were fractionated, then dried and stored under the

conditions described in Table III. When the fractionated rubber was dried under air, the viscosities of the first two fractions were lower than when dried under nitrogen as shown by the values obtained after no days of storage. During storage, the least change in the viscosities occurred when the rubber was stored under nitrogen in a refrigerator. Very definite reductions in viscosities were obtained when the rubber was stored in air in the diffuse light of a room. Again, it was the highest molecular weight fractions that showed this loss of viscosity in benzene solution. These results indicate that the rubber films should be dried under a stream of nitrogen and that they then can be stored for at least a week in an atmosphere of nitrogen without any deterioration or change in the molecular weight of the rubber.

TABLE III

INHERENT VISCOSITIES OF RUBBER FILMS OBTAINED FROM REPLICATE FRACTIONATIONS DRIED IN A VACUUM OVEN AT 30° C UNDER DIFFERENT CONDITIONS AND THEN STORED IN DESICCATORS FOR DIFFERENT LENGTHS OF TIME UNDER VARIOUS CONDITIONS BEFORE REDISSOLVING IN BENZENE

TREATMENT AND TIME OF STORAGE	VISCOSITY OF RUBBER IN INDICATED FRACTION						PROPORTIONAL AVERAGE
	1	2	3	4	5	6	
Dried in air, stored in air at room temperature and light*							
0 Days	6.58	4.30	3.38	2.75	2.14	1.40	5.48
3 Days	6.42	4.33	3.28	2.70	2.17	1.45	5.37
7 Days	6.00	3.84	3.38	2.73	2.28	1.20	5.03
Dried in nitrogen, stored in air at room temperature and light*							
0 Days	6.90	4.80	3.49	2.76	2.13	1.37	5.53
3 Days	6.64	4.85	3.48	2.70	2.26	1.42	5.39
7 Days	5.85	4.44	3.21	2.61	2.23	1.40	4.82
Dried in nitrogen, stored in nitrogen at room temperature and light*							
0 Days	6.95	4.61	3.48	3.04	2.16	1.45	5.58
3 Days	6.82	4.70	3.55	3.08	2.08	1.48	5.51
7 Days	6.75	4.56	3.59	3.10	2.37	1.52	5.47
Dried in N_2, stored in N_2 at 5° C and darkness							
0 Days	6.81	4.77	3.39	2.93	2.13	1.45	5.57
3 Days	6.85	4.59	3.40	2.90	2.31	1.44	5.59
7 Days	6.80	4.54	3.43	2.94	2.40	1.44	5.55

* By "light" is meant the diffuse light in a room, not direct exposure to the sun.

After the rubber had been redissolved in benzene, the question arose as to how long the solutions could be held without suffering a change in their viscosity. To test this, benzene solutions from the various fractions were stored in a dark room at 20° C in glass-stoppered volumetric flasks and the viscosities and concentrations determined after 0, 7 and 60 days. No significant change in the inherent viscosities of the rubber occurred after

seven days although some slight loss was noted after 60 days (table IV). This meant that the solutions could be held for a few days before determining their viscosities without introducing errors. It also shows that over a long period of time the rubber molecules tend to break down under such conditions of storage.

It will be noticed in tables I and II that very often slightly more than 100% of the rubber estimated to be in the solution before fractionation may

TABLE IV

THE INHERENT VISCOSITIES OF BENZENE SOLUTIONS OF RUBBER STORED FOR DIFFERENT LENGTHS OF TIME IN THE DARK AT 20 DEGREES CENTIGRADE

TIME OF STORAGE	INHERENT VISCOSITY OF RUBBER FROM INDICATED FRACTIONS						
	UNFRACTIONATED	1	2	3	4	5	6
DAYS							
3	6.42	6.70	5.50	3.85	3.61	2.51	1.61
7	6.43	6.66	5.45	3.86	3.61	2.49	1.58
60	6.39	6.53	5.32	3.65	3.53	2.40	1.52

be accounted for when the amounts recovered in each fraction are totaled. This is, of course, too high, especially as very small quantities of rubber remain in solution after the sixth fraction has been precipitated. The rubber is estimated in the original sample by brominating, but only as total solids in the fractions. As the total solid content of the unfractionated rubber solution was always greater than the amount of rubber determined

TABLE V

PERCENTAGE OF TOTAL SOLIDS IN VARIOUS FRACTIONS RECOVERED AS BROMINATABLE RUBBER

UNFRACTIONATED	1	2	3	4	5	6
91.6 ± 2.1[1]	$99.8 \pm .4$	$99.7 \pm .4$	$99.8 \pm .1$	$99.8 \pm .8$	$100.7 \pm .7$	99.5 ± 1.1

[1] = Standard error of the mean based on 8 replications.

from bromination, it seemed possible that on fractionation nonbrominating material might be carried down with the precipitating rubber. However, as shown in table V, practically all the material precipitated in the various fractions could be accounted for as brominatable rubber, even though only 92 per cent of the total solids in the unfractionated solutions could be recovered as rubber bromide.

The first investigation carried out with this technique was to determine the differences in the fractional distribution and the molecular weight of the unfractionated rubber and of that in the various fractions found in different parts of the plant. Random samples of different aged shrub growing in different areas in the vicinity of Salinas were gathered and

divided into various parts and the fractional distribution of their rubber determined. The results of two such determinations are shown in figures 1 and 2. The values are the means of five replications of three plants each. The tips consisted of the current year's growth; the branches, of the parts between the tips and the main trunk; the stems, of this above-ground trunk; and the roots, of the underground parts to a depth of six to eight inches. In the older plants (fig. 1) the branches were again split into younger and older parts.

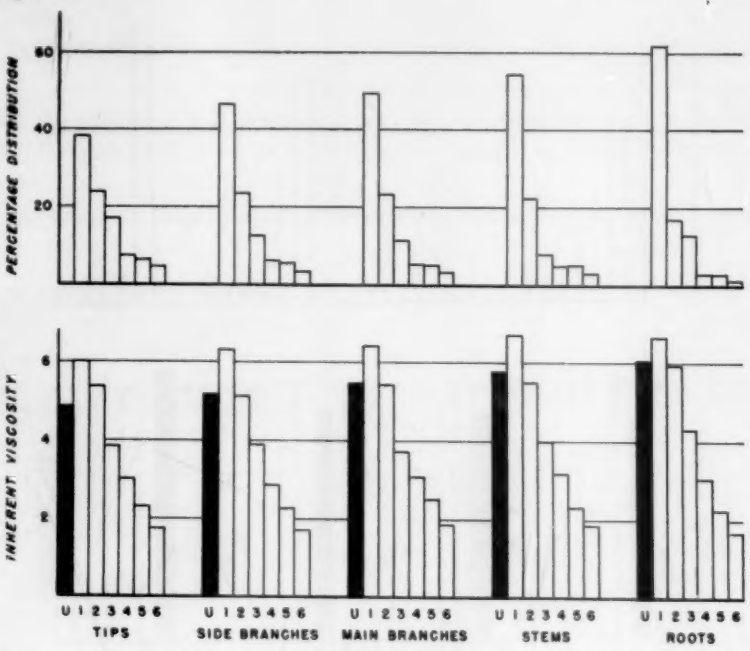

FIG. 1. Percentage distribution and inherent viscosity of unfractionated rubber (U) and of the various fractions (one to six) of the rubber obtained from different parts of 13-year-old guayule shrub, Strain 593, from Spence Field.

Proceeding downward along the plant, the inherent viscosities of the unfractionated rubber (U) increased, being lowest in the tips and highest in the roots. The fractionation data show that this increase was not so much due to the increased viscosity of the various fractions but to the increased amount of rubber recovered in the first or highest molecular weight fraction. The viscosity of the first fraction was generally somewhat higher in the roots than in the tips or branches. Regardless of the plant part, the viscosities of the last three fractions (fourth, fifth, and sixth) did not differ greatly from each other. Most of the rubber was found in the first and second fractions, while the percentage of the fifth and sixth fractions was particularly small.

In spite of the differences in age and time of harvest of the two collections of shrub, the fractional distribution and viscosity of the rubber did

not differ greatly between them. Other data to be published elsewhere show that season of the year and climatic conditions can influence the character of the rubber in the plants.

Olson (4) has shown that the crude rubber milled from the roots of guayule is inferior in quality to that milled from the above-ground parts of the plant. When the above results were obtained showing the highest molecular weight rubber to be in the roots, the criticism of the method was made that the highest molecular weight rubber in the above-ground

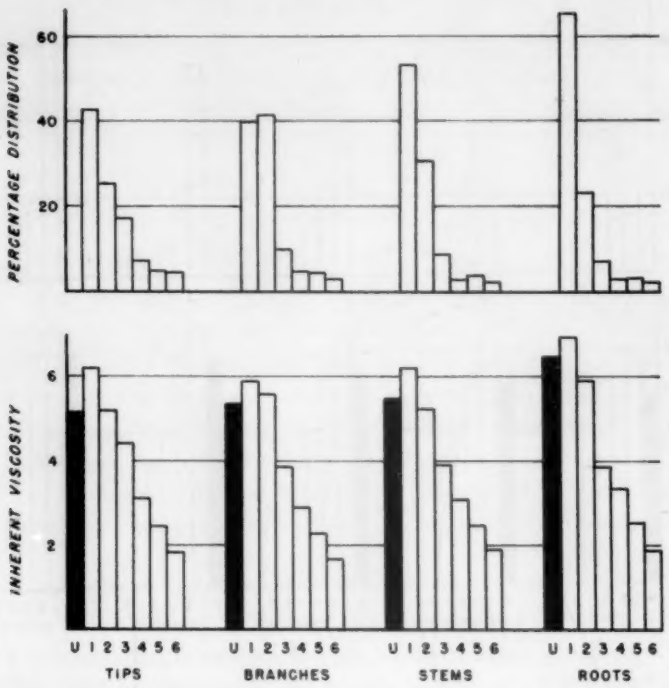

Fig. 2 Percentage distribution and inherent viscosity of rubber from 5-year-old guayule shrub, Strain 593, grown in Arguello Field. Legend same as for Figure 1.

parts was not extracted or that only the higher molecular weight rubber in the roots was extracted. To check on this point, random samples of 4-year-old shrub were harvested in September 1948. The shrub was divided into tips, branches, stems and roots, their rubber content determined (7) and then extracted according to the method described. The bagasse was saved and the remaining rubber determined. This bagasse was again extracted with acetone and benzene and the amount of rubber obtained in the second extraction was determined. The fractional distribution and viscosities of the rubber in the various fractions were determined on both the first and second extracts. From these determinations, it was possible to show how much of the original rubber was removed from the plants by the first and then the second extractions (table VI) and also how the frac-

TABLE VI

THE WEIGHT AND PERCENTAGE OF THE RUBBER RECOVERED FROM TWO SUCCESSIVE BENZENE EXTRACTIONS OF DIFFERENT PARTS OF GUAYULE SHRUB

PLANT PART	ORIGINAL CONTENT OF SHRUB	RECOVERED IN FIRST EXTRACTION		RECOVERED IN SECOND EXTRACTION		TOTAL RECOVERED IN TWO EXTRACTIONS	
	gms.	*gms.*	*%*	*gms.*	*%*	*gms.*	*%*
TIPS	13.81	12.05	87.3	.96	6.9	13.1	94.2
BRANCHES	15.74	12.64	80.3	2.55	16.2	15.19	96.5
STEMS	14.31	12.04	84.1	1.70	11.9	13.74	96.0
ROOTS	7.98	5.14	64.4	2.37	29.7	7.51	94.1

tional distribution and viscosity data of the first extraction would have been changed if it had included that obtained in the second extraction (fig. 3).

The results (table VI) show that the standard procedure of extraction

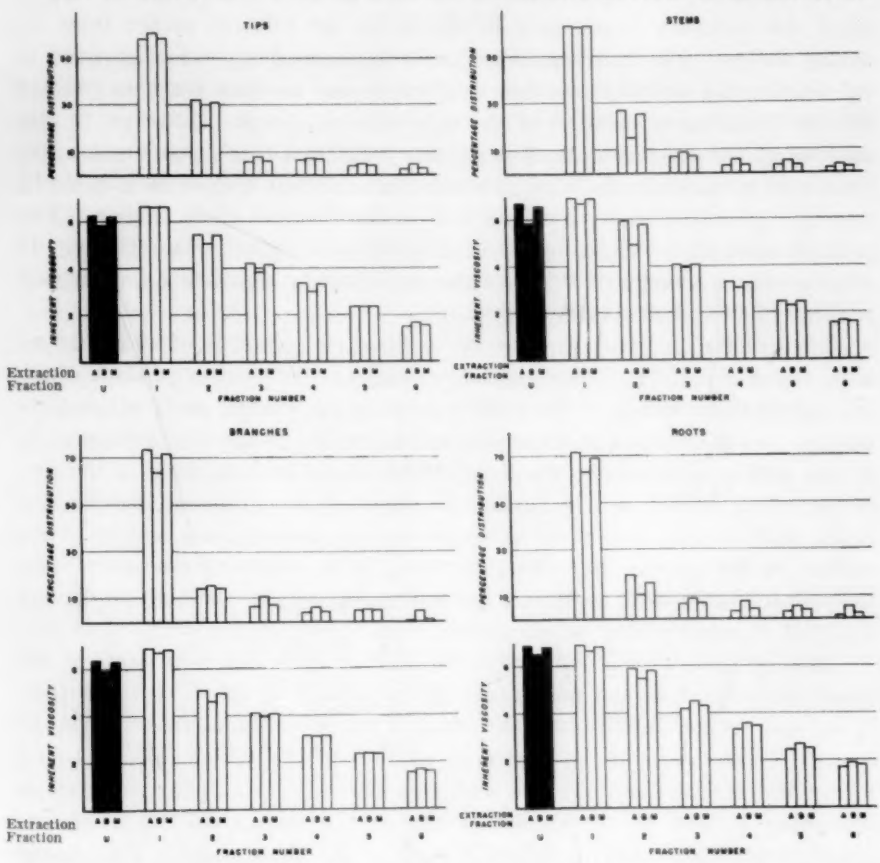

FIG. 3. Percentage distribution and inherent viscosities of the rubber in the various fractions obtained from the first benzene extraction of various parts of guayule shrub (A), the second extraction (B), and the proportional average of the two (M).

removes more than 80% of the rubber in the above-ground parts of the plants and more than 60% of that in the roots. A second extraction with benzene, for the same length of time, removed all but 6% or less of the rubber from the plant tissues. From figure 3, it can be seen that the fractional distribution and viscosity readings of the fractions obtained from the second extraction may differ slightly from those obtained from the first extraction. It can also be seen that the weighted means of the first and second extractions do not differ greatly from the results obtained from the first extraction alone. Hence, the conclusion drawn from results obtained by the standard procedure described would not be altered by a somewhat more complete extraction of the rubber. Thus, the data on this sample of shrub still show that the roots had the highest molecular weight rubber and the tips, the lowest.

Discussion

In discussing the significance of the data presented, it should be emphasized that primary importance is placed on the relative rather than the actual values. For this reason and also because of the error involved in calculating the actual molecular weights, it was not necessary to proceed further than the calculation of the inherent viscosities. However, to give an idea of the range of actual molecular weights under consideration, the data used to calculate the highest and lowest inherent viscosities in table II, namely 8.05 and 0.98, were substituted in the formula given earlier. This yielded molecular weight values of 210,000 and 20,000, respectively. In other words, a change of 1.00 in inherent viscosity represents a change of roughly 27,000 in molecular weight.

The results, in general, show the method described is of sufficient accuracy and reliability to demonstrate changes or differences in the molecular weight distribution of the rubber between, or within parts of, guayule plants. As the method does not subject the rubber to any heat treatment or to any milling process, the results obtained should be indicative of the condition of the rubber as it exists in the plant. Thus, data can be obtained which will indicate any effect of treatment on the molecular weight of the rubber in the plant. For example, data to be reported elsewhere show that considerable shift occurs in the molecular weight distribution during a period of rapid rubber accumulation.

Most of the differences between the rubber from different parts of the plants are found in the percentage and viscosity of the first three fractions, and greater differences are found in the percentages than in the viscosity. In the roots of the plants, 60 to 80% of the rubber may be found in the first fraction while in the stem tips around 40%. The viscosities or molecular weights of the rubber of the first fractions do not differ too greatly from each other in different parts of the same plant. This means that the higher molecular weight of root rubber is due largely to the fact that a greater proportion has been built up into the highest molecular

weights fraction rather than that the actual molecular weight of the rubber is any greater.

The highest inherent viscosity yet found in guayule has been 8.65. The rubber in the plant seems to build up to a molecular weight giving such an inherent viscosity and then no further increase occurs. This high value was obtained in a refractionation of a first fraction. JOHNSON (**3**) reports inherent viscosity values of over 13.00 for Hevea rubber and shows in one sample over 35% of the rubber to yield inherent viscosity values of 11.00 and above. SAUNDERS and SMITH (**5**) state that the infrared "Spectra of the purified elastomers are nearly identical and serve to confirm the belief that the polymeric units are the same for these three elastomers" (Hevea, guayule, and kok-saghyz). From this it is interesting to speculate on the differences between the plants which result in the rubber reaching a much higher molecular weight in one than in the other.

Summary

A method is described whereby resin-free rubber from guayule plants can be extracted without subjecting it to heat or milling. Procedures are also given for fractionating this rubber into various portions of differing molecular weights and estimating the percentage of each fraction and relative values of their molecular weights from the viscosity of dilute solutions in benzene.

From results obtained using the above techniques, it has been shown that the rubber in the roots of guayule had the highest molecular weight, that of the stems the next to the highest, followed by that of the branches, and that of the tips being the lowest. The results also show that the first or highest molecular weight fractions show the same trend with different parts of the plants, and that a much greater proportion of the rubber from the roots is found in the first fraction than from the stem tips.

The highest molecular weight estimate obtained for any fraction of guayule rubber was not so high as some reported for Hevea rubber.

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ON THE THEORETICAL INTERPRETATION OF TURGOR PRESSURE

T. C. Broyer

(WITH ONE FIGURE)

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In a recent publication, Burström (**5**) has endeavored to "evaluate the physical meaning of the turgor pressure of a living cell." Modifications have been suggested in the schematic representation of osmotic quantities with change in volume of the cell, from that originally proposed by Thoday (**7**) and modified by Höfler (**6**). He has included a curve representing the influences of solute in the external medium bathing the cell, approximately paralleling the curve representing the influence of solute in the internal medium. The net effect of the osmotic influences (at any particular volume of the internal medium) related only to the solute differences of the internal and external media, is thus satisfactorily represented. A similar inclusion was made by Broyer (**1**), but represented in a different manner, *viz.*, a line approximately paralleling the curve usually representing the influence of an internal hydrostatic pressure. In the latter, the loci of the curves allow a more ready summation of the scalars representing influences causing water to tend to move in similar directions (inward or outward) through the cytoplasmic membrane of the cell.

It is usually recognized that, under conditions of osmotic disequilibrium such that a net influx or efflux specific free energy (**1**) exists, a finite but infinitesimal difference obtains between the internal hydrostatic pressure (turgor pressure in the usual sense) and the wall pressure. This is caused by the net movement of water across the interposed differentially permeable membrane separating the internal and external media, and attended by a corresponding change in the volume of the internal phase. There is a progressive infinitesimal, correlated change in each of the factors with time, concomitant with water movement, tending to approach osmotic dynamic equilibrium as a limit. Attended by metabolism and growth, there is a continuous shifting of the equilibrium determined by current environmental factors within limits imposed by the genetic potentialities of the organism.

Burström has defined turgor pressure in a sense quite distinct from the common usage, *viz.*, "turgor pressure equals the difference in osmotic values of cell sap and external solution." He regards turgor pressure to be an actual, instantaneously and fully effective pressure "acting from inside on the cell wall," i.e., "the expanding power of the cell content," at any particular time. He visualizes turgor pressure and wall pressure to be "of fundamentally different physical origin, and each may attain any value in-

dependent of the other." (See figure 1) He suggested (using his symbols and wording[1]) that

$$T = O - E \tag{1}$$

and

$$O = S + W \tag{2}$$

are two independent relationships among the osmotic quantities. He combined these equations, solving for $T - W$ (by substituting $S + W$ from equation 2, for O in equation 1), and obtained the relation

$$T - W = S - E. \tag{3}$$

"These two formulas (1 and 2)[2] are independent of each other. If the cell is not at equilibrium, $S \gtrless E$ and $T \gtrless W$. It also follows that under all circumstances $T - W = S - E$. That means that if a cell is absorbing water owing to a difference in suction $(S - E)$ there must also exist an excess of turgor pressure over the wall pressure $(T - W)$. T equals W only if the cell is at equilibrium with the external solution."

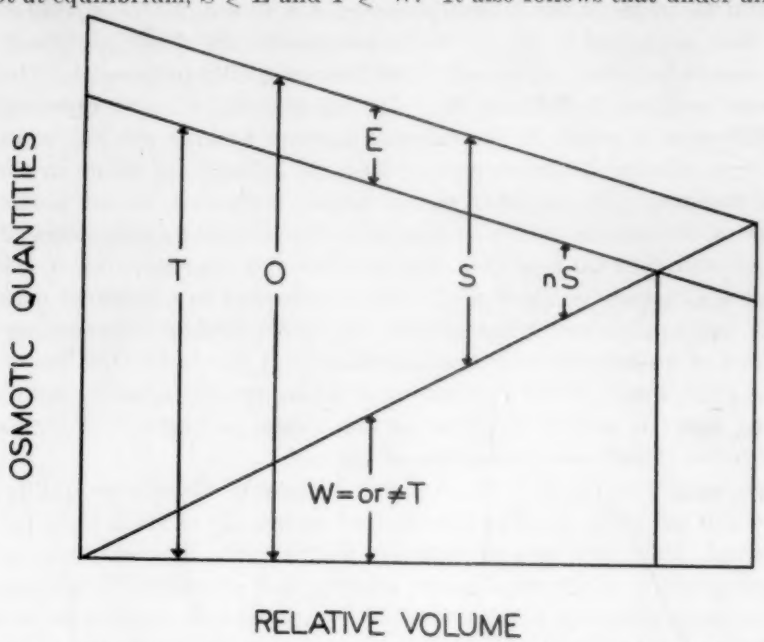

FIG. 1. Diagrammatic relationship among various osmotic quantities and the relative volume of the internal phase of a cell osmometer. In the usual osmotic diagram $T \tilde{=} W$. The de Vries-Burström concept visualizes T to be disjunct from W, as represented otherwise above.

DE VRIES (9) may have had a concept similar in part at least to that suggested by Burström, *viz.*, ". . . der Zellsaft muss bei der Aufnahme von

[1] Here "T is the turgor pressure, O is the osmotic value of the cell sap, E is the osmotic value (or "suction") of the external medium, W is the wall pressure, and S is the suction of the entire cell."

[2] Author's insert.

Wasser den Widerstand der elastisch gedehnten Zellhaut überwinden, und die wasseranziehende Kraft des ganzen Gewebes ist also gleich der Turgorkraft seiner Zellsäfte, vermindert mit der elastischen Spannkraft der Zellhäute."—page 544. (See equation 5.)[3] He also stated, "Die Wasser anziehende Kraft des turgescenten Gewebes ist nun zwar nicht dieselbe die des in seinen Zellen enthaltenen Zellsaftes, sondern um so viel geringer als der elastischen Spannkraft der Protoplaste und der Zellhäute entspricht. . . ." —page 437 (equation 2 or 4).

Assuming the usual concept of turgor, the single equational expression (equations 2 or 4, or 6) including all necessary internal and external influences of an osmotic system (compare (1) equation 4a and 8), would seem to indicate the osmotic relations quite satisfactorily of itself. Herein, turgor pressure (or better, hydrostatic pressure) and wall pressure are often substituted one for the other because of their generally recognized approximate equality under all conditions. Burström proposes that "turgor pressure is not caused by a net diffusion of water, nor by an increase in volume and a hydrostatic pressure (compare 4, pg. 24)[2] as in an osmometer, but only by the (net)[2] diffusion pressure (restricted to effects caused by a difference of solution concentration in the two media bathing the interposed membrane)[2] of the water molecules." This latter viewpoint, and the combination of equations 1 and 2 leads to a concept which is quite thought prompting. Here, as with the strict definition of osmotic pressure (1, pg. 5), an osmotic pressure difference can be realized at any instant of time—the biological and physical systems considered to be similar. Assuming acceptance of the defi-

[3] Detailed comparative analysis might be as follows:

I. Burström's equations, including E in equation 1 and omitting the same in equation 2, S representing the suction of the cell;

$T = O - E$ (1)

$O = S + W$, and (2)

substituting $S + W$ from equation 2, for O in equation 1,

$T = S + W - E$, or

$T - W = S - E$ (3)

II. Alternative equations, where the external medium is water (i.e., omitting E in both equations), nS representing the net suction by the cell;

$T = O$ (1a)

$O = nS + W$, and (4)

substituting $nS + W$ from equation 4 for O in equation 1a,

$T = nS + W$; (5)

or, where the external medium is a solution (i.e., including an E in both equations), nS representing the net suction by the cell;

$T = O - E$ (1)

$O = nS + W + E$, and (6)

substituting $nS + W + E$ from equation 6 for O in equation 1,

$T = nS + W + E - E$, or

$T = nS + W$ (5)

If the alternative analysis may be assumed to represent the viewpoint of de Vries, then, the net water absorbing "force" of the cell relative to the external medium (nS) would be involved here rather than the suction of the cell *per se* (S) as used by URSPRUNG (8) and BURSTRÖM (4, 5).

nition of turgor as a resultant "rigidity in living cells, caused by pressure of the water contents against the elastic cell membrane," it might appear unwise however, to redefine turgor pressure as the net osmotic value (net osmotic specific free energy related only to the presence of solute in the internal and external media), equal to such an osmotic pressure difference. Under practical conditions, rigidity change, involving finite time, is usually conceived as associated with a net movement of water and corresponding change of volume of the inner phase. Therefore, it seems questionable whether any advantage accrues from grouping any certain limited number of factors causing water to tend to move in a particular direction other than, for example, the sum of the influx intensities (1),[4] at any particular time.

It is difficult to reconcile Burström's postulate that "turgor pressure must decrease when a cell absorbs water and the wall pressure increases," with the usual viewpoint. Further, under conditions appropriate to his concept, it might be interpreted that complete "turgor" expansion or contraction should be realized merely in the time necessary for membrane (including the wall) stretching or shrinking, since the "pressure" here would be fully effective at any condition of non- or dynamic equilibrium and independent of water movement as a causal factor. The alternative usual concept of turgor pressure, in accord with experimental observation, requires an extended measurable time for approach toward full realization of the turgor potentialities, accompanied by a progressive movement of water. Here, the time involved is that necessary for the net migration of water across the membrane and that required for the concurrent stretching or shrinking of the limiting surface.

Burström suggested that the osmotic diagrams of Höfler and Tamiya[5] are valid only at water equilibrium. This scheme, first proposed by Thoday and generally used, seems to be acceptable for all situations where all factors (E, etc., also) are included (compare (1) fig. 3 and equation 4a).

A detailed analysis of common ideas on the movement of materials into plants has been presented (**1, 2, 3**).[4] A discussion of some experimental observations on the water relations of roots will be reported elsewhere.

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[4] At the suggestion of others, it would be wise for dimensional accuracy, to recognize all flux specific free energies as practically measurable translational energy intensities (here volumed) rather than "action capacities."

[5] Referred to by Burström. Actually, Tamiya's representation of turgor pressure, based on experimental data of Stow with *Nitella*, follows that theoretically suggested first by Höfler, compare BROYER (1).

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TRANSLOCATION OF EXOGENOUS GROWTH-REGULATORS IN THE BEAN SEEDLING

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(WITH THREE FIGURES)

Received June 27, 1949

Introduction

Localized presentation of growth-regulatory chemicals to a particular organ of a plant can give rise to responses in other, more or less distant, parts, a circumstance that may be taken as evidence of the translocation of a stimulating substance which may be termed the "stimulus." In most instances there is no information at hand to permit a decision as to whether the translocated stimulus is the applied chemical itself, a derivative of the applied compound, or some endogenous hormone, the formation or movement of which has been influenced by the treatment. In a few cases, however, there is rather clear-cut evidence that the applied compound is transported as such (**6, 8, 17**) and at present there seems to be no cogent argument to gainsay that this may be universally true.

It has been shown (**7**) that the stem curvature response of bean seedlings to leaf application of 2,4-dichlorophenoxyacetic acid requires the exposure of the plants to relatively intense light and carbon dioxide concurrently. Such exposure need not take place while the plant is responding but is effective even though terminated some time before application of the growth-regulator. The response itself may occur in absence of light or of CO_2. No stem curvatures occur in leaf-treated plants which have been maintained in darkness, or in light of low intensity, for several hours preceding treatment, or in plants which have been exposed to bright light in a CO_2-free atmosphere. However, under such environmental conditions, plants do give stem curvature responses to growth-regulators applied directly to the stem, to the cotyledons, or to the roots. Furthermore, under conditions in which leaf presentations do not lead to stem curvatures, there may occur localized torsions of the lamina in the vicinity of the applications.

Conversely, a latent response may be elicited by holding treated carbohydrate-depleted plants in darkness for a considerable period and then exposing them to conditions favorable to photosynthesis as shown by the following experiment. Seven-day-old bean seedlings (*Phaseolus vulgaris* var. Black Valentine) were placed in a dark room for 24 hours after which a 0.01 ml. drop containing 10 micrograms of ammonium 2,4-dichlorophenoxyacetate in 1% aqueous Carbowax 1500 was placed on one primary leaf of each plant. Groups of the treated plants were transferred from the darkroom to the greenhouse at intervals up to 72 hours. No responses, other than localized leaf torsions, were evident while the plants were in

darkness but all exhibited characteristic stem curvatures within a few hours after exposure to light. Control plants treated in the same manner, except for having the treated portion of the leaf removed just prior to transfer from dark room to greenhouse, showed no growth-regulator response.

These facts indicate that the favorable influence of light is not directly upon the curvature (growth) response itself or upon the entry of growth-regulator into the plant, but rather upon movement from the leaf blade to and through the stem. The experiments of RICE (**9**, see especially fig. 4) indicate that entry into the leaf is actually greater in darkness than in light even though export from the carbohydrate-depleted leaf does not occur under the former condition. It has been suggested by various investigators that translocation of the growth-regulatory stimulus from the leaf is associated with concurrent transport of organic food materials produced by photosynthesis. Evidence consistent with this view has been presented by the demonstration that the magnitude of response induced by leaf application was correlated with the sugar content of the leaf blade (**7**), and was virtually prevented by drastically decreasing the leaf area available for photosynthesis (**15**). It was shown also that movement of the stimulus from the leaf took place only through living cells.

Role of nutrients in growth-regulator transport

The following experiments were undertaken to ascertain whether translocation of applied growth-regulators might be influenced by specific food materials artificially supplied to the leaf. That sugar can enter an intact leaf from solutions in which it is immersed and be utilized for shoot development in darkness has been shown for various species (for literature see **16**).

In general, the technique employed was as follows. Bean seedlings were cultured in soil in the greenhouse until the second internode had begun to elongate (usually eight days after planting). The cotyledons were removed by gentle pressure with the fingers and the plants were placed in darkness for 24 or more hours in order to deplete the leaves of reserve carbohydrates. Subsequent manipulations were conducted in light of low intensity (5 foot candles or less), the duration of illumination being kept as short as possible. All darkroom experiments were conducted at approximately 26° C. Under these conditions there was very little stem elongation in unfed plants. One or both of the primary leaves were gently rolled and inserted into vials of 30 to 60 ml. capacity into which the desired solutions (unsterilized) were then introduced. All liquids supplied to the leaves contained 0.025% sulfanilamide, as used by WENT and CARTER (**16**), for the purpose of minimizing microbial development. The sulfanilamide appeared to be without influence upon the beans, as shown by: (1) comparison of sucrose solutions containing 0.025 and 0.05% sulfanilamide, (2) an experiment in the greenhouse in which equal shoot growth was made

during a 5-day period by untreated plants and by those with one primary leaf immersed in aqueous sulfanilamide solution, and (3) comparison of sugar solutions with and without sulfanilamide in shorter tests (two to three days) during which microbial contamination was not apparent.

Figure 1 presents results of an experiment in which stem elongation varied linearly with concentration of supplied sucrose up to 0.75 M. In this concentration range the 2-day increment was very nearly two fifths that in five days, indicating that the growth rate was substantially constant. Proportionality between growth and sugar concentration has been observed repeatedly, although the absolute amount of growth obtained with a particular concentration may vary considerably from experiment to experiment unless the plants are very carefully selected for uniformity.

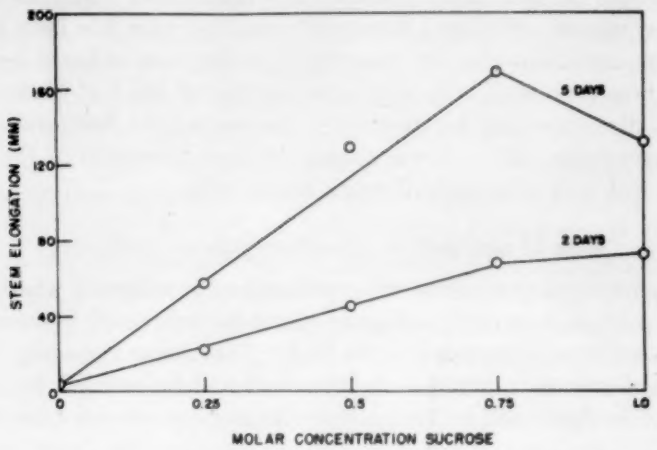

FIG. 1. Shoot elongation in darkness as influenced by concentration of sucrose supplied to leaf. Each point represents the mean of seven plants.

Glucose and maltose are approximately equal to sucrose in supporting growth, whereas fructose is somewhat inferior; lactose and galactose are much less satisfactory, possibly owing, in part at least, to the leaf injury which they induce. Whether extracellular hydrolysis of sucrose must precede absorption as reported by SAÏD (**11**) has not been ascertained.

The growth response to sucrose was not increased by buffering the solution with phosphate at pH 4.6 or 5.7; it was reduced nearly to half at pH 6.6, and was completely abolished at pH 8.8. This result indicates also that sugar absorption was not limited by the phosphorus content of the leaves as may occur with P-deficient leaves according to SAÏD (**11**). Addition of 0.1% or 1% Tween 20 to the unbuffered sucrose solution more than doubled its effectiveness.

Elongation of the stem thus may be taken as evidence that a supplied substance, or its conversion product, is transported from the leaf to the growing region. The amount and rate of growth may be influenced, of

course, by factors such as ease of penetration into the plant, rate of transport, and intrinsic suitability of the supplied compound as a nutrient or its convertibility into such.

Growth of treated plants may be either increased or decreased relative to untreated controls, depending on whether small or large amounts of the exogenous growth-regulator are present in the growing region.

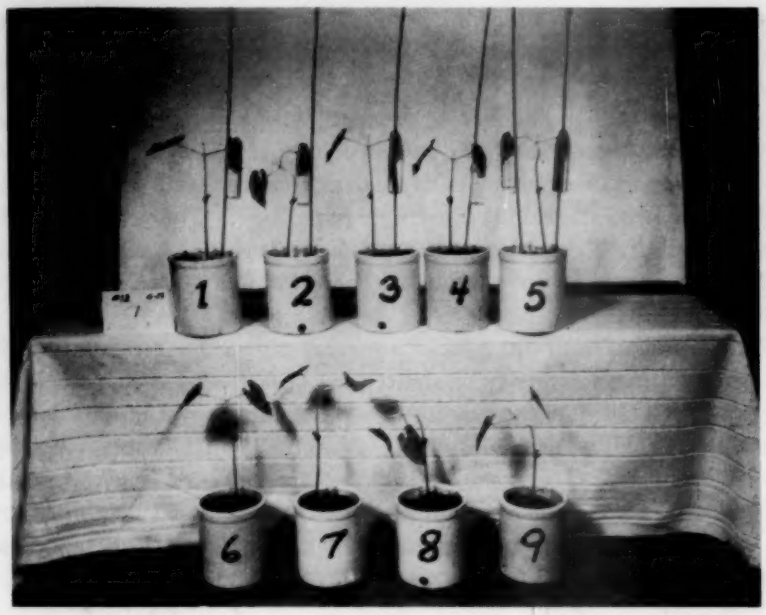

FIG. 2. Effect of sucrose on response to 2,4-dichlorophenoxyacetic acid. Unless otherwise specified, plants were depleted of carbohydrate by 24-hour dark period preceding treatments and maintained in darkness for additional 24 hours prior to photographing.

1—Leaf immersed in 1 M sucrose.
2—Leaf in 1 M sucrose plus 50 p.p.m. 2,4-D.
3—Leaf in water.
4—Leaf in 50 p.p.m. 2,4-D.
5—Right leaf in 1 M sucrose; left leaf in 1 M sucrose plus 50 p.p.m. 2,4-D. (Initial curvature towards right.)
6—Untreated.
7—10 micrograms 2,4-D applied as aqueous solution (0.01 ml.) to right leaf.
8—Treatment as in No. 7 without prior depletion of carbohydrate.
9—1 microgram 2,4-D applied unilaterally to first (epicotyledonary) internode.

SCHUFFELEN (**12**) has speculated that growth-regulators in high concentration may impede sugar transport by decreasing protoplasmic permeability. We incline to the view that the diminished growth observed with sugar plus growth-regulator in the present study is due primarily to an inhibitory effect of the latter upon some process more directly involved in

growth, rather than to an interference with the sugar supply to the meristem.

Because small stimulations would be difficult to detect in these experiments it was more convenient to employ relatively high concentrations of growth-regulator so as to utilize inhibition as an indicator of its movement from the leaf. Further demonstrations of transport are furnished by curvature of the stem, which results from the unilateral distribution of growth-regulator in this organ following application to a single leaf, and by hypertrophy of petiole and stem which sometimes ensues.

As indicated by these criteria it has been demonstrated that, in the absence of light, exogenous growth-regulators are translocated from a starved leaf to which they are supplied only if nutrient (sugar) also is furnished to the same leaf. A typical experiment with sucrose and 2,4-dichlorophenoxyacetic acid is illustrated in figure 2. There was no evidence of movement of the growth-regulator from the leaf if it were applied alone, or if growth-regulator were applied to one leaf and sugar concurrently to the opposite leaf.

Similar results were obtained with several other growth-regulating chemicals representing a diversity of structural types: indole-3-acetic acid, beta-hydroxyethyl-4-chlorophenoxyacetate, p-chlorophenyl-2,4,5-trichlorophenoxyacetate, 4-chlorophenoxyacetamide, 2-bromo-3,5-dichlorobenzoic acid, N-(2,4-dichlorophenoxyacetyl)-urea, and the morpholine salt of 2,4-dichlorophenoxyacetic acid. Hence it appears that the effect of sugar upon transport of a growth-regulating compound is not dependent upon the presence in the latter of any particular molecular grouping.

In addition to sucrose, the following sugars have been found to facilitate movement of growth-regulators: glucose, fructose, maltose, lactose, and galactose. As shown by the shoot development, all of these sugars also serve as nutrients, although it is not established whether they are transported and utilized in the form supplied or first undergo some transformation. In table I are presented results from two experiments which show the comparative growth-promoting efficacy of several sugars. While these are typical of several other experiments, only the relative order of magnitude is to be regarded as significant. It is of interest that galactose is utilized to some extent inasmuch as this sugar has been found non-available for growth of other tissues of higher plants although respirable and convertible into starch (**1, 2, 10, 14**).

The non-specificity with respect both to growth-regulator and to sugar suggests that transport of the stimulus is facilitated by the occurrence of food movement in general rather than by any specific combination with a particular metabolite. It may be postulated that movement of the stimulus from the leaves of bean seedlings is dependent upon the operation of the mechanism responsible for translocation of organic materials but that the

growth-regulator itself is unable to initiate the transport process. Despite much work during the past several decades, the means by which organic materials are moved in plants remains one of the puzzling problems of plant physiology. Elucidation of the mechanism of movement of growth-regulators may require the solution of this more general problem. Insofar as the present results bear on this question they seem to point toward some type of mass conduction. It should, perhaps, be stated that these conclusions are not intended to apply to growth-regulator transport in specialized organs such as the grass coleoptile.

It would be of considerable interest to determine whether substances other than sugars can set the train of conduction in motion and so promote

TABLE I

ELONGATION IN DARKNESS OF SECOND INTERNODE OF BLACK VALENTINE BEAN SEEDLINGS SUPPLIED BY LEAF IMMERSION WITH SOLUTIONS OF SUGARS AND 2,4-DICHLOROPHENOXYACETIC ACID. (ALL SOLUTIONS CONTAIN 0.025% SULFANILAMIDE. TEN REPLICATES FOR EACH SOLUTION.)

EXPT.	SUGAR (0.25M)	TWEEN 20 (%)	ELONGATION (MM/PLANT)		
			2,4-D (P.P.M.)		
			0	10	25
1	None	0.1	1	1	.....
	Sucrose	0.1	48	4	.....
	Glucose	0.1	46	4	.....
	Maltose	0.1	41	4	.....
	Fructose	0.1	29	4	.....
	Lactose	0.1	8	3	.....
2	Lactose	.....	4	.....	2
	Galactose	.....	6	.....	2

transport of growth-regulators. Conversely, the use of growth-regulators as indicators of movement of various supplied materials might constitute a helpful tool for determining which compounds are translocated in plants, a subject upon which there seems to be relatively little unequivocal information.

Routes and mechanisms of growth-regulator transport

Various pathways and correspondingly diverse mechanisms of transport of the growth-regulatory stimulus in plants may be operative depending upon circumstances.

PHLOEM TRANSPORT

Growth-regulators which enter the plant through the intact epidermis of stems or leaves may move either acropetally or basipetally, or in both directions concurrently.

If the amount of growth-regulator applied to the intact leaf is relatively small, downward transport appears to be restricted to living cells, presumably those of the phloem. This conclusion is based on the non-con-

duction basipetally through a killed region of petiole or stem. In such experiments tissue proliferation, and sometimes root formation, occur just above the killed zone, a phenomenon which may be taken as evidence that the stimulus has been transported via phloem elements and has accumulated at the site of the responses. Conduction of stimulus by the phloem of squash has been reported to approach a velocity of 20 cm. per hour (**13**).

The consensus of opinion at present appears to favor the phloem as the principal route of conduction of organic nutrient materials from the leaf. Accordingly, it may be inferred that this is the path principally involved in growth-stimulus movement of the type influenced by sugar. This view is supported by the results of experiments in which solutions were supplied, as described above, to intact leaves, the petioles of which had been flamed. Neither sugar nor growth-regulator, alone or in combination, appeared to traverse the killed zone under these conditions.

TABLE II

EFFECT OF FLAMING FIRST (EPICOTYLEDONARY) INTERNODE ON ACROPETAL TRANSLOCATION FROM PRIMARY LEAF OF BLACK VALENTINE BEAN SEEDLINGS

SUPPLIED TO UNIFOLIOLATE LEAF	FIRST INTERNODE	ELONGATION OF SECOND INTERNODE (MM)
Water	intact	1
	flamed	1
Sucrose	intact	23
	flamed	16
Sucrose + 2,4-D	intact	3
	flamed	8

The leaf-feeding technique has provided further information on the route of conduction from the primary leaf of the bean seedling. According to the anatomical studies of DOUTT (**3**), the three principal bundles supplying the unifoliolate leaf make direct connection with ascending major bundles in the stem, not at the level of the primary-leaf node, but near the level of the cotyledonary node. If this is correct, it might be expected that materials originating in the leaf and destined for the more apical regions of the shoot would move first downward at least as far as the cotyledonary node and then upward. Such a route would explain the curvatures in the internode below the primary leaf node which commonly result from application of growth-regulator to one of these leaves in seedlings at the stage described above.

The data in table II indicate that the amounts of sugar and of growth-regulatory stimulus transported from the primary leaf to the shoot apex are indeed diminished if a dead zone is present in the epicotyledonary internode, and thus are not inconsistent with the pathway described. However, inasmuch as conduction from leaf to apex is not prevented completely by the existence of the dead region, it is probable that an alternative route of movement is available.

Xylem transport

A quite different mechanism of transport is involved when growth-regulators find their way into the transpiration stream, as by absorption through the roots or by injection into the shoot. In such case, movement is controlled by those environmental factors which influence transpiration and may take place through a killed zone (**4, 5, 13**). Conduction in this manner, which in general is acropetal, is considerably more rapid than that in the phloem, velocities as great as 70 cm. per hr. having been noted (**13**).

Entry of 2,4-D and/or sugar into the plant via the xylem can be accomplished also by immersing the debladed end of a petiole into an appropriate solution. Separately or in combination these substances can pass through a flamed segment of a petiole, as shown by the previously de-

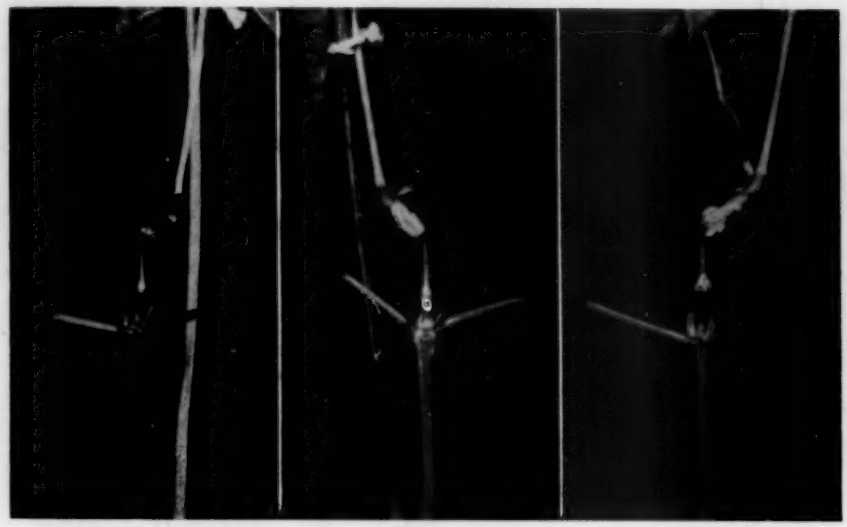

Fig. 3. Movement of 2,4-D stimulus through flamed region in second internode as indicated by stem proliferation. (Leaf blades and axillary shoots removed for photograph.)

Left—Flamed only. No growth-regulator.

Center—Both primary leaves below flamed zone painted with aqueous solution containing 1000 p.p.m. 2,4-D and 1% Tween-20.

Right—0.1 ml. aqueous solution containing 10 micrograms 2,4-D and 1% Tween-20 applied to first trifoliolate leaf above flamed region.

scribed criteria. If a solution containing both 2,4-D and sugar is presented to a debladed petiole, stem curvature usually occurs while shoot elongation tends to be suppressed. The entry of these materials is probably due to a reversal of the normal transpiration stream, so that movement is away from rather than toward the leaf.

Application of large doses to the intact primary leaf may be followed by acropetal movement through a killed region of the stem as evidenced by

the tissue responses (fig. 3). In this case movement from phloem to xylem appears to have occurred.

Summary and conclusions

Growth-regulating chemicals applied to the leaf of a bean seedling devoid of translocatable carbohydrates are not transported from the leaf to the stem. Movement of such growth-regulators can be brought about by supplying sugar to the leaf externally. A variety of sugars (sucrose, maltose, lactose, glucose, fructose, galactose) are able to induce translocation. Growth-regulators of diverse structural types (indole-3-acetic acid, β-hydroxyethyl-4-chlorophenoxyacetate, p-chlorophenyl-2,4,5-trichlorophenoxyacetate, 4-chlorophenoxyacetamide, 2-bromo-3,5-dichlorobenzoic acid, N-(2,4-dichlorophenoxyacetyl)-urea, 2,4-dichlorophenoxyacetic acid, and its morpholine salt) are transportable, no particular molecular grouping being requisite. The non-specificity with respect to both sugar and growth-regulator appears inconsistent with the occurrence of a definite combination between the two. Rather, movement of the growth-regulator seems to require only that the normal mechanism of transport of organic materials be set in operation by whatever means.

Growth-regulator translocation of the type facilitated by sugar takes place only through living cells, presumably of the phloem. A quite disparate mode of conduction occurs when the growth-regulator is introduced into the transpiration stream. The latter may be carried on through non-living regions of stem or petiole and is independent of movement of sugars.

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INFLUENCE OF TEMPERATURE ON THE VITAMIN CONTENT OF GREEN PLANTS[1]

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Change in the temperature with seasons is such an important factor in plant growth that its influence on the vitamin content of plants deserves a thorough investigation. Yet very few such investigations have been made and all but one of these have been concerned with ascorbic acid. One of the peculiarities of these investigations has been that they do not agree as to the effect of temperature on the concentration of ascorbic acid in plants. HAMNER *et al.* (**5**) found that tomato fruits produced at a temperature of 78° F (26° C) had a higher ascorbic acid content than fruits produced at 63° F (17° C), while ÅBERG (**1**) states that young tomato plants grown at 15.5° C had a 30% higher content of ascorbic acid than plants grown at 23° C. REID (**8**) found a lower concentration in cow pea seedlings grown at 29° than at 24° C and MOLDTMANN (**7**) noted that 10-day-old *Vicia Faba* seedlings had nearly twice as much ascorbic acid at 26° as at 14° C, whereas *Pisum sativum* seedlings had a much lower vitamin content at 26 than at 14° C. *Avena sativa* seedlings showed no essential difference at the two temperatures. SOMERS *et al.* (**10**) working with discs of turnip leaves floated on nutrient solution found that a higher concentration of ascorbic acid was obtained in discs grown at 24° C than in discs grown at 14° C. RODAHL (**9**) states that *Empetrum nigrum* has a higher concentration of ascorbic acid in northeast Greenland than in southeast Greenland. Presumably the former region has a lower temperature than the latter. MITCHELL and HOULAHAN (**6**) reported a mutant of Neurospora which in order to make satisfactory growth has to be supplied with riboflavin at 28° C but not at lower temperatures. WOLF (**11**) found that excised leaves of *Bryophyllum calycinum* lost ascorbic acid at a temperature of 20° but not at 7° and 37° C.

Materials and methods

It will be noted in the above review of the literature that aside from Moldtmann the different investigators used only one species of plants. In this investigation 10 species have been used and these have ranged in age from about two weeks to seven months. The following plants have been used: Early Jersey Wakefield cabbage, Soybean, Black Valentine beans, Green Sprouting broccoli, Red clover, *Lupinus albus*, Alaska peas, John

[1] Paper from the Department of Botany of the University of Michigan, No. 891. This investigation has been aided financially by the Horace H. Rackham Trust Fund of the University of Michigan and the U. S. Public Health Service, and some of the chemicals have been supplied by Merck and Company, Inc.

Baer tomato and 4n Ex-haploid tomato, Giant Thickleaved spinach, and a variety of winter wheat. Only stems and leaves have been used. When a number of experiments were run with a variety such as tomato, broccoli cabbage and clover, plants of different ages were used and leaves from different parts were also employed in different experiments in order that the results might be a composite of the plant. The number of plants used in an experiment varied somewhat but generally material was taken from 10 to 20 plants except in one experiment where only one large cabbage plant was available in each temperature. In evaluating these experiments it must be taken into account that plants of a particular species were used from all temperatures on the same day and in the same experiment. When this was done different samples of the same plant varied only 1 to 3%. Great care was always exercised to take material of the same stage of development in comparable experiments. Plants were exposed to the temperatures 10–15° and 28–30° C in two large greenhouse compartments with an east exposure. An intermediate temperature of 20–22° C was also provided and in a few experiments, plants from this temperature were also used, though as a rule this temperature was used only for growing the seedlings and storing the plants until they were placed in the experimental rooms. In some of the preliminary experiments, the plants were exposed to the desired temperature for only one or two days but these were not duplicable and in the experiments reported here the plants were held at least a week and usually several weeks or even months at the temperature designated.

In general, the plants were grown in a larger greenhouse off the campus until of a considerable size and ready to be used, but some of the plants that were used when only a few weeks old were ordinarily grown in the intermediate temperature house. Sometimes seedlings were started at the intermediate temperature and as soon as they were several inches high, they were transferred to the experimental temperatures. The lower temperature retarded the development so much that after one or two weeks the plants were not of the same developmental stage and could not be used for comparative purposes. When this happened the plants were shifted to the opposite temperature and used a week or two later. In this way, two lots of plants of the same calendar age but of different physiological age were obtained, and the ones from the higher temperature might be used several weeks before those from the cooler temperature.

The low temperature was maintained by keeping the thermostat set for 10° and the windows partly open. This arrangement was effective only when the temperature outside was considerably below 10°. This investigation has extended over two winter seasons (1947–48 and 1948–49).

The vitamins, thiamin, riboflavin and niacin (nicotinic acid) have been determined in the plants under study. The two former were determined chemically according to the method of Connor and Straub as adapted by the writer (**3**) for green plant material. The niacin has been determined

by the microbiological method as outlined in the Methods of Vitamin Assay (**2**), with some minor changes necessitated by the materials used.

During the latter part of the investigation all three vitamins have been determined on each lot of plants. For each assay, a composite sample made from a number of plants was prepared, whenever material was available, though in a few instances only one large plant may have been used, and then leaves and stems from the different parts were used if it was impossible to use the whole plant. The plant material was macerated by a Waring blendor. For the thiamin and riboflavin determinations, an aliquot of 50 ml. of this blend was autoclaved for 15 minutes at 15 pounds of pressure and incubated with 10 ml. of a 10% clarase solution at 37° C for about 24 hours. The digested mixture was then centrifuged and the supernatant liquid used as previously indicated in making the assays (**3**). For the niacin assay a 40 ml. aliquot was made acid with 5 ml. of six Normal H_2SO_4, autoclaved for 30 minutes at 15 lbs. pressure, neutralized to pH 6.8, diluted to the proper concentration, filtered and the proper amount used in the various assay tubes. For the usual run of material, dilutions were made to eight grams of plant material per liter of solution and this solution was used at the rate of 1, 2, 3, 4 and 5 ml. per tube of 10 ml. of solution. In preparing the material with the Waring blendor, 4 ml. of distilled water were used for each gram of plant material and after maceration, 5 ml. of 0.1 Normal H_2SO_4 were added for each gram of plant material. Thus the final mixture contained one gram of plant material per 10 ml. of blend. When an abundance (50 to 100 g.) of plants was available, the blend was permitted to settle and the clear liquid pipetted out, but when smaller amounts of material were used, filtering was resorted to in order to avoid getting bits of plant material.

Results

During the winter of 1947–48, the investigation was begun with mature plants of John Baer tomato and a 4n Ex-haploid tomato. Tips of the plants or mature, but not old, leaves were used in all of the experiments with the tomato. As table I indicates, the three vitamins were higher at the higher temperature. This was expected from Hamner's work with tomato fruits (**5**). At this time, Åberg's paper on tomato leaves was not known to the writer. During the first winter, several experiments were performed with beans, but in most of them, young seedlings were placed at different temperatures and when the plants were large enough to be used in the warm room, they were not as far developed at the lower temperature, and therefore not comparable as to physiological age and only two of these experiments with beans are presented in this report. In these experiments, the plants were of the same stage of development in both temperatures, though of a different chronological age. At this time, the niacin determination was not satisfactory enough and the data for niacin are

TABLE I

INFLUENCE OF TEMPERATURE ON THE CONCENTRATION OF THIAMIN, RIBOFLAVIN AND NICOTINIC ACID IN GREEN PLANTS. THE FIGURES REPRESENT VITAMIN AS MICROGRAMS PER GRAM OF FRESH PLANT MATERIAL. A MINUS IN FRONT OF THE FIGURE IN COLUMN "% INCREASE IN COLD" DENOTES THAT THERE WAS A HIGHER CONCENTRATION IN THE PLANTS AT THE HIGHER TEMPERATURE THAN AT THE LOWER ONE, WHEREAS A PLUS DENOTES A HIGHER CONCENTRATION IN THE LOWER TEMPERATURE

PLANT	NO. OF EXP.	THIAMIN			RIBOFLAVIN			NICOTINIC ACID			NO. EXP. WITH NIACIN
		27–30	10–15	% INCR. IN COLD	27–30	10–15	% INCR. IN COLD	27–30	10–15	% INCR. IN COLD	
Beans	2	1.18	1.06	−13	1.84	1.51	−22				0
Broccoli	5	0.76	0.88	+16	1.24	1.58	+27	7.06	9.23	+30	4
Cabbage	5	0.86	1.06	+23	1.33	1.55	+16	8.33	9.03	+ 9	3
Clover	6	1.50	1.51	0	2.48	2.77	+12	8.05	9.78	+21	2
Lupines	2	0.93	1.21	+30	1.69	1.96	+16	6.90	6.70	− 3	2
Peas	5	1.44	1.36	− 6	1.65	2.00	+24	9.91	10.27	+ 4	3
Soybean	3	1.41	1.13	−20	2.41	2.06	−14	7.32	6.90	− 6	3
Spinach	2	1.25	1.33	+ 6	1.61	1.84	+14	3.83	6.30	+61	2
Tomato	9	1.25	0.86	−45	1.41	1.33	− 6	7.21	6.01	−20	6
Wheat	4	1.31	1.29	− 1.5	2.17	2.69	+24	6.92	10.43	+50	3

therefore not included. In the bean the thiamin and riboflavin concentrations were also higher at 28–30° than at 10–15° C. In the fall, several cabbage plants had been lifted from the field, potted and brought into the greenhouse, where they were exposed to different temperatures. In February, thiamin and riboflavin were determined in these plants and surprisingly enough it was found that the plants in the cold room had a much higher concentration of both vitamins than the plants from the warmer room. The plants in the warm room were not as healthy looking as those in the cold room, but still the leaves used were all in good condition. Two experiments with mature clover plants were also run, but the results were rather indecisive. In one experiment, the thiamin was slightly higher in the plant from the cold room and in the other, slightly higher in the plants from the warm room, but in both experiments, the riboflavin was higher in the plants from the cold room. This experiment necessitated the adoption of a hypothesis that the different vitamins might not be affected by the temperature to the same extent in different plants. This has since been verified substantially. Three experiments with peas were also performed during the first season. The thiamin was again variable, but the riboflavin was always more abundant in plants from the low temperature.

In the more or less preliminary experiments of the first season, tomato, bean, cabbage, clover and peas had been used. It had been established that not all plants respond to the same extent to the temperature in their vitamin synthesis nor are all vitamins affected in the same way. An inspection of the data suggested that plants making their best growth during the warm part of the summer had a higher vitamin content at the higher temperature, whereas plants like cabbage, clover and peas that grow better during the cooler part of the summer, and in spring and fall, tended to have a higher vitamin content at the lower temperature; and that thiamin is likely to be higher at the higher temperature and riboflavin lower at the higher temperature. The niacin determinations were too few and incomplete to enable one to formulate an opinion concerning their response.

As a result of the first season's experiments, cabbage, broccoli and spinach, which are cool season plants, were prepared for the second year. Seeds of these were planted early in August and in the fall, when the experiments on the effect of temperature were again taken up, large plants were available. It was also learned that too young seedlings could not be used, as prolonged exposure to different temperatures produced plants of different stages of development. It is necessary to obtain fairly mature plants first, preferably grown at an intermediate temperature, then transfer them to the desired temperature and leave them there from a week to several weeks.

By the time the work was begun the second season, the niacin assay method had been mastered and it was determined on all plant lots except in some where difficulty was encountered in the analysis. All three vita-

mins were then determined during the second season. To the five species used during the previous season were added broccoli, spinach, white lupine, soybeans, and wheat. As the general procedure was the same as that earlier discussed, there is no need to give details of each experiment and each kind of plant. The composite data of all experiments are presented in table I.

The John Baer and the 4n Ex-haploid tomatoes have been combined as there was no difference in the results. The most striking feature of the table is the fact that only three species, tomato, beans and soybeans, had a higher vitamin content at the 28–30° temperature than at the 10–15° temperature. Broccoli, cabbage and spinach can be put in a group by themselves as showing a higher concentration of all three vitamins at the lower temperature, just the opposite of the tomato, bean and soybean plants. Clover, lupines, peas and wheat must be thought of as intermediate between the other two groups. On the whole, the vitamin content in this group is higher at the lower temperature, but in three of them, the thiamin is either higher at the 28–30° temperature or the same at both levels, and in one, niacin is higher at the higher temperature. The only one that is consistently present in a higher concentration at the 10–15° is riboflavin. Looking at the table from the standpoint of the individual vitamin rather than from the plant angle, it is evident that the thiamin content is more favorably influenced by a high temperature, as in five plants it is higher at the 28–30° and in one plant there is no difference and in only four plants is it lower at the higher temperature. Contrasted with this is the riboflavin which is present in a higher concentration at the lower temperature in seven out of ten plants. Niacin seems also to be more abundant at a low temperature.

One suggestion that can be offered as an explanation is that plants that make their best growth at the high temperature of the summer also synthesize more vitamin at a high temperature, and any other temperature which is not so favorable for growth is likewise not favorable for the vitamin synthesis. Then plants which grow during the low temperature periods of the year also synthesize or at least are richer in vitamins at those periods. Several experiments with tomato plants in the dark and at different temperatures show that in the dark at 5° C, the decrease in vitamins is much less than at higher temperatures. In a previous paper (**4**), the writer has shown that plants use up their vitamins in the dark. In the present experiment, is was found that the plants in the 28° temperature darkroom lost much more vitamin than those at 5° C. Therefore we might assume that certain plants have a higher vitamin content at the lower temperatures than at higher ones because at the lower temperature there is less loss than at higher temperatures, rather than an increase in synthesis.

This investigation does explain why in the past different investigators have obtained divergent results. Not only have they used widely different temperatures, but also a variety of plants of different ages, though most of the investigators used seedlings. In this investigation, 10 different plants have been used and at some times three different temperatures.

To the practical minded individual, be he a plant grower or nutritionist, these experiments should offer some help. Knowing the temperature at which the vitamin is highest in a plant, it can be used most widely during that period. Not only will this keep one supplied with an abundance of vitamins but it will also tend to supply one with a variety in the diet.

Summary

This investigation has dealt with determinations of the vitamins, thiamin, riboflavin and niacin (nicotinic acid) in the vegetative parts of a number of plants subjected to the temperatures of 10–15° and 28–30° C.

It has been found that all three vitamins are more abundant in tomato, bean and soybean at the temperature of 28–30° C than at 10–15° C.

Broccoli, cabbage and spinach on the other hand, are richer in these vitamins at the lower temperature.

In clover, peas, and wheat, the thiamin content was higher at 28–30° C, though the riboflavin and niacin were more abundant at the lower temperature.

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FURTHER STUDIES ON THE CHEMICAL INDUCTION OF PARTHENOCARPY IN THE CALIMYRNA FIG

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(WITH FOUR FIGURES)

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Introduction

During the summer of 1947 investigations were initiated to find chemicals which would induce parthenocarpic development of second-crop Calimyrna figs, and thus relieve the grower of the problems of caprification. In that preliminary work three growth-regulating chemicals were employed, namely, 2,4-dichlorophenoxyacetic acid, beta-naphthoxyacetic acid, and gamma-(indole-3)-n-butyric acid. At the concentrations tested only the latter compound was effective. The purposes, problems, and details of this preliminary study have been reported (**3**).

With the experience gained from the 1947 studies the work was expanded in 1948 with considerable success, and the results are presented here.

Materials and methods

The investigation was primarily concerned with the testing of several synthetic growth-regulating compounds to determine their effectiveness in promoting parthenocarpy in second-crop Calimyrna figs. However, other studies incidental to the main program also were made which will be discussed later.

The methods employed were essentially similar to those used in the original work, differing only in minor details. The investigations were conducted in commercial orchards under standard practices of production. Materials under test were applied with a continuous delivery type, hand-operated fly-spray gun, wetting both leaves and syconia with the spray. Individual branches were used and were selected for uniformity of vigor and number of syconia. Five branches were used per treatment, each bearing at least five syconia at the time of treatment. To exclude the pollen-carrying fig wasp (*Blastophaga psenes* L.) these branches were covered with muslin bags during the period of caprification. A total of 50 branches selected at random throughout the orchard served as controls and were caprified by the grower, along with the rest of the orchard, in the accepted commercial fashion. Ten additional branches were bagged to serve as controls with no caprification or chemical treatment.

All test branches were bagged on June 24 and with the exception of a few minutes during treatment remained covered until July 12. Caprification was started in the orchard on June 25 and the last introduction of caprifigs was made on July 5, 1948. All spray applications were made on June 30 and July 1, 1948.

Nine chemicals at a number of concentrations were tested, both alone and in various combinations. In all, 83 treatments were made which represented a total of 475 branches that were observed through the growing season. Table I lists the treatments employed.

Chemicals in the acid form were dissolved in distilled water with the aid of a minimum quantity of ammonium hydroxide, following which the pH of the solution was adjusted by the addition of HCl to between 5.0–5.5.

TABLE I

CHEMICALS AND CONCENTRATIONS TESTED TO DETERMINE THEIR EFFECTIVENESS IN PROMOTING PARTHENOCARPY IN THE CALIMYRNA FIG

CHEMICAL USED	CONCENTRATIONS (P.P.M.)				
Sodium 2,4-dichlorophenoxy acetate (2,4-D)	20	40	60	80	100
Isopropyl ester of 2,4-D in oil emulsion (2,4-DE)	20	40	60	80	100
2,4,5-trichlorophenoxyacetic acid (2,4,5-T)	10	25	50	75	100
Isopropyl ester 2,4,5-T in oil emulsion (2,4,5-TE)	10	25	50	75	100
para-chlorophenoxyacetic acid (PCPA)	10	20	40	60	80
Sure-Set*	22	33	44	67	
alpha-(2-chlorophenoxy) propionic acid (alpha 2)	50	100	150	200	
alpha-naphthaleneacetic acid (NA)	25	50	100	250	
Endrop*	25	50	100	250	
beta-naphthoxyacetic acid (NOA)	100	250	500	750	
Indole-3-acetic acid (IA)	25	50	100	250	
Indole-3-propionic acid (IP)	50	100	250	500	
gamma-(Indole 3)-n-butyric acid (IB)	200	400	600	900	1200 1500
NA 125 p.p.m. plus 2,4-D at =	20	40	60	80	100
NOA 125 p.p.m. plus 2,4-D at =	20	40	60	80	100
NOA 125 p.p.m. plus PCPA at =	10	20	40	60	80
NA 125 p.p.m. plus PCPA at =	10	20	40	60	80

* Sure-Set, supplied by the Dow Chemical Company, contains PCPA in a water-miscible solvent, and Endrop, supplied by Shell Oil Company, Incorporated, contains NA in a light emulsive oil.

The sodium salt of 2,4-D was dissolved directly in water and the pH adjusted as above. The isopropyl esters of 2,4-D and 2,4,5-T were dissolved in a light emulsive oil which was then emulsified by vigorous shaking with the proper amount of water so that the final spray contained 1.0% oil.

Results and discussion

The following compounds were completely ineffective in inducing parthenocarpy at the concentrations employed: 2,4-D, 2,4-DE, NOA plus 2,4-D, alpha-2, NOA, IA, and IP. The negative results obtained with 2,4-D and NOA are in agreement with the previously reported work (**3**). However, it was found that 100 p.p.m. of 2,4-D did not cause injury to the tree and it is possible that higher concentrations may be effective in inducing parthenocarpy.

Those treatments that effectively induced parthenocarpy are set forth in table II, along with the percentage of the treated syconia that matured and the average air-dry weight of the ripe fruit.

The data show that NA, 2,4,5-T, IB and PCPA were effective in inducing parthenocarpy in the Calimyrna fig. The use of an oil emulsion with NA increased the activity of the material, but only at the three lower concentrations. Increasing concentrations of both NA and PCPA caused an increase in per cent. fruit set within the range of concentrations used here.

TABLE II

PER CENT. FRUIT SET AND AVERAGE AIR-DRY WEIGHT OF FRUIT PRODUCED BY VARIOUS TREATMENTS

MATERIAL	CONC. P.P.M.	PER CENT. SET	AVERAGE DRY WEIGHT GRAMS/ FRUIT	MATERIAL	CONC. P.P.M.	PER CENT. SET	AVERAGE DRY WEIGHT GRAMS/ FRUIT
2,4,5-T	10	56	.....	Endrop	25	39	14.1
	25	64			50	72	
	50	69			100	72	
	75	72			250	80	
	100	65					
2,4,5-TE	10	60	.....	IB	200	23	13.4
	25	77			400	56	
	50	83			600	76	
	75	63			900	69	
	100	67			1200	78	
					1500	79	
PCPA	10	4	17.2	NA 125 plus	20	77	15.1
	20	18		2,4-D at:	40	83	
	40	67			60	58	
	60	76			80	70	
	80	62			100	50	
Sure-Set	22	58	14.6	NA 125 plus	10	76	14.4
	33	76		PCPA at:	20	79	
	44	46			40	88	
	67	46			60	78	
					80	54	
NA	25	8	16.4	NOA 125 plus	10	19	12.9
	50	42		PCPA at:	20	36	
	100	63			40	74	
	250	85			60	77	
					80	67	
Caprified Controls	A*	45	16.9	Untreated-uncaprified		0	
	B*	83					

* A—Controls in orchard where sprays were applied. B—Controls in a nearby orchard.

PCPA apparently was most effective at about 60 p.p.m. The acid and ester forms of 2,4,5-T showed somewhat the same trend, although the data are more variable. The combination of two chemicals did not greatly affect the results, the per cent. fruit set being about equal to that obtained with the most active material alone.

Although NA, 2,4,5-T, and IB were effective in promoting parthenocarpy

they were also toxic to the tree in varying degree. NA produced little or no injury at the lowest concentration but severe chlorosis at 250 p.p.m., the concentration at which it was most effective in stimulating parthenocarpy. The same symptoms were apparent when NA was used at 125 p.p.m. in combination with other materials. At concentrations of 75 and 100 p.p.m., 2,4,5-T, under these conditions, caused death of the treated branches in about a month. However, this material produced some unusual effects and will be discussed in detail below. IB, in agreement with the previous work, was moderately toxic at concentrations of 600 p.p.m. or more. Chlorosis was the typical symptom, although some leaf epinasty was evident.

FIG. 1. Fig branches bearing mature fruit. Right, caprified control, left, parthenocarpic fruits induced with 60 p.p.m. para-chlorophenoxyacetic acid.

On the other hand, PCPA was a highly effective compound without any observable symptoms of injury. Figure 1 illustrates branches of caprified and PCPA parthenocarpically induced fruits at maturity.

The significance of the injury produced by these chemicals is somewhat difficult to evaluate because the sprayed branches were enclosed within muslin bags for a period of 19 days, 12 of which were after the sprays were applied. Just what influence the bagging might have on the susceptibility of the branches to chemical injury is not known, but it is possible that such a treatment might accentuate injury through reduced light intensity and also by confining vapor from the applied chemicals. This seems particularly true of 2,4,5-T, since branches adjacent to those treated with the 100 p.p.m. preparation received small quantities of spray as drift and yet showed very little injury, even though many of the fruits responded similarly to those in the experiment.

The percentage of sprayed fruit that reached maturity in the successful treatments was far greater than in the caprified controls in the orchard in which the work was done. However, when compared to the percentage of fruit set in a near-by orchard, the set on the sprayed branches was slightly lower. This difference in control data is due simply to differences in thoroughness of caprification. The same method was followed in each case, but the frequency of placing caprifigs and the number used were different. The percentage fruit set of the controls in both orchards have been included in order that valid comparisons could be made.

All the fruit produced by chemical stimulation was completely devoid of achenes ("seeds"), and there was no sclerification of ovary walls. The

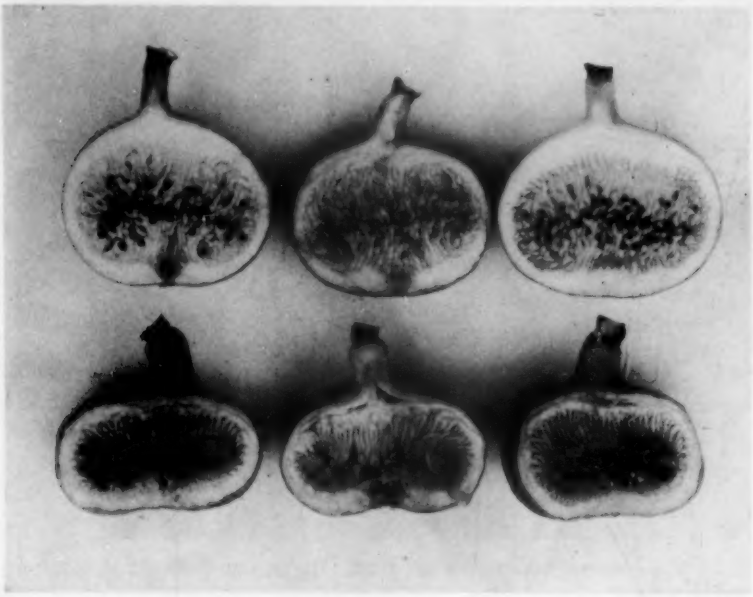

FIG. 2. Sectional views of caprified fruit (upper) and parthenocarpic fruit (lower) induced by 60 p.p.m. para-chlorophenoxyacetic acid.

flavor and color of the fresh fruit was the same as that of the caprified controls. There was a difference in shape between the parthenocarpic and caprified fruit, the former being somewhat flattened from top to bottom. Figure 2 illustrates this difference.

The sugar content of the mature PCPA produced fruit was 51% of the dry weight and that of the mature caprified fruit 46%. Despite the lack of achenes, the average air-dry weight per fig produced by NA and PCPA was essentially equal to that of the controls. IB produced fruit that was about 79% of the weight of those caprified, although equal in diameter.

Diameter measurements of fresh mature fruits were made only in the case of IB, PCPA, and controls. IB at 1200 and 1500 p.p.m. produced fruit of 51 and 52 mm. average diameter, respectively, PCPA at 60 and 80 p.p.m. 55 and 56 mm. respectively, and the controls, orchard A and B, 53 and 51

mm. respectively. All other parthenocarpic fruits, although not measured, were observed to be normal in size.

Periodic diameter measurements were made of developing fruits of the controls and those produced by IB and PCPA. The growth curves were practically alike as to general form, and are typified by those previously published (**3**), except that the PCPA-induced fruits matured at the same time as those caprified.

All parthenocarpic fruits were combined into a common lot and processed and packed in the usual commercial manner. There was insufficient fruit for experimentation on processing methods so all fruit was treated exactly like commercial caprified fruit. The resulting pack was of good appearance and equal in weight to normal fruits. A number of taste tests were made of the processed material, and, although the flavor was considered acceptable in most instances, it was generally agreed that the flavor was milder and somewhat different from the caprified fruit. It is believed that this difference was due in part to the processing method, since the flavor of dried parthenocarpic fruit before processing was comparable to dried caprified fruit, and in part to the lack of the essential oils of the achenes. The "nutty" texture of the normal fruit was of course lacking in the parthenocarpic material.

As a result of this season's work it is believed that a solution is in sight for the Calimyrna fig grower's problems of caprification. Because of its relatively low cost, low required dosage, and lack of injurious effects, parachlorophenoxyacetic acid appears at this time to have much promise as a material to induce parthenocarpic fruit development in the Calimyrna fig. More work must be done on the problem before any general recommendations can be made, but the results to date are very encouraging.

In addition to results obtained on the original practical problem, interesting fundamental aspects have developed. It was mentioned above that 2,4,5-T produced some very unusual results. Syconia that were sprayed with preparations of this material, either in the acid form or as the ester in an oil emulsion, reached maturity, as judged by color, size, texture, and taste about two weeks after treatment. Normally, such syconia when caprified or treated with other effective growth-regulators require about 75 days in which to attain a comparable stage of maturity. (See figure 3, curves A & C). This stimulation of the maturation process was found at concentrations as low as 10 p.p.m. of the acid or ester, but lower concentrations of the acid were not effective. The fruit thus produced was completely devoid of achenes.

The stimulating effects were not confined to those syconia which were receptive to pollination (or to parthenocarpic stimulus by other hormones) but were obtained on all syconia on the branch. Even the very young syconia, which would not have been receptive to pollination for a week or more after the time of spraying increased somewhat in size, became yellow, soft, and sweet.

Following the initial discovery, sprays of 2,4,5-T were applied to caprified fruits on July 29. Here it was found that the lowest effective concentration was 25 p.p.m., as contrasted to 10 p.p.m. for uncaprified syconia. The caprified fruits reached maturity in about two weeks although they normally would not have matured for another 30 days. (See figure 3, curves B & C.)

Further trials were made with the acid at 20 p.p.m. on fruits of the Black Mission variety. Since the spray was applied considerably later in

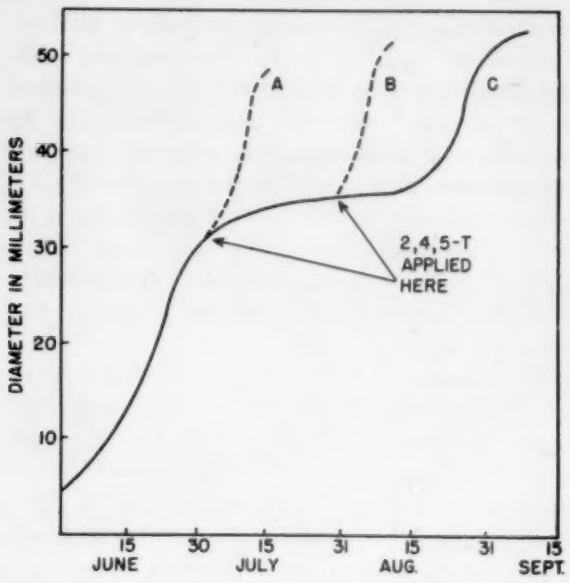

FIG. 3. Curves of growth in diameter of unpollinated (A) and pollinated (B) Calimyrna fig fruits sprayed with 25 p.p.m. 2,4,5-trichlorophenoxyacetic acid as compared to pollinated but unsprayed control fruits (C). Curves represent the average diameters of the four proximal-most figs on current season's growth.

the season, the difference in time of maturity between the sprayed and unsprayed fruit was not as pronounced as with the Calimyrna. The treated Black Mission fruits matured 17 days ahead of the untreated controls (see figure 4).

The rapid acceleration of the fruit maturation processes by the application of a synthetic plant hormone is considered to be of considerable fundamental significance and has been discussed in a previous publication (**2**).

The data obtained in this work emphasize the high degree of specificity of the synthetic growth-regulators in regard to the reactions they induce. Three very closely related compounds—PCPA, 2,4-D, and 2,4,5-T—produced widely different results. PCPA induced parthenocarpic fruit which developed in a manner very comparable to caprified fruit; 2,4-D was completely ineffective in the concentration range used; and 2,4,5-T induced parthenocarpic development at an extremely rapid rate. While it is believed that

these diverse reactions are actually the result of differences in the manner in which the synthetic materials alter the normal chain of events leading to maturation the effect of such factors as rate of penetration into the plant and translocation within the plant cannot be overlooked.

It is of interest to compare the results of these chemicals on the fig with data of ZIMMERMAN and HITCHCOCK (7) on the induction of parthenocarpy in the tomato. Of the six chemicals common to both sets of data, three, IB, PCPA, and 2,4,5-T, are effective on both fruits in about the same concentration ranges, whereas the other three, 2,4-D, NOA, and alpha 2, are ineffective on the fig at concentrations five to 10 times that necessary for the tomato. The reason for these differences in activity is not immediately ap-

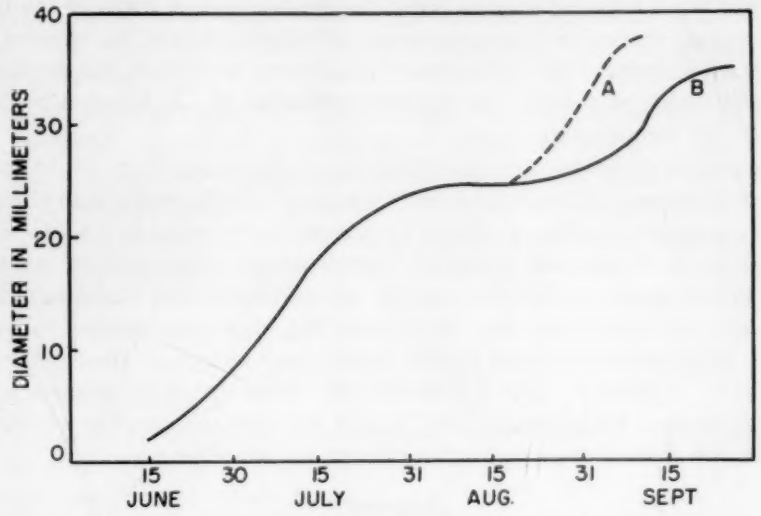

FIG. 4. Curves of growth in diameter of Mission figs sprayed with 20 p.p.m. of 2,4,5-trichlorophenoxyacetic acid on August 17, 1948. Sprayed fruits (A) and unsprayed control fruits (B).

parent but the great difference in fruit type is no doubt largely responsible.

The necessity for translocation of growth-regulators to regions of reactivity was illustrated by means of IB. This material at concentrations of 1200 and 1500 p.p.m., was applied to either syconia or leaves only. When applied to the leaves typical injury occurred, indicating penetration, but parthenocarpy did not result. However, application to syconia alone did induce parthenocarpic fruit growth to maturity without leaf injury. Apparently under the conditions of this experiment, IB was not translocated from the leaves to the syconia in sufficient quantity to induce parthenocarpy. The "transmission of effect" as found by Batjer (1) for NA on apple drop clearly did not occur in the above experiment.

The fig offers considerable promise as a test plant for studies on the hormone relations of fruit development. Complete natural parthenocarpy occurs in several varieties, such as Adriatic, Black Mission, and Kadota,

whereas the Calimyrna is practically completely non-parthenocarpic. In the latter variety a few syconia of the first crop may develop parthenocarpically, while the second crop is completely non-parthenocarpic. In addition, parthenocarpy can be induced in second crop Calimyrna figs with PCPA, resulting in a normal rate of fruit development, or with 2,4,5-T, where an accelerated rate of development occurs. Furthermore, the maturation of caprified Calimyrna or parthenocarpic Black Mission fruits can be greatly accelerated with 2,4,5-T. Thus it seems possible that studies of the hormone relations of fruit development in the fig might yield valuable information on fruit development in general.

It is clear that the source of hormones for fruit development to maturity is not in the developing embryo, since the parthenocarpic fruits of the Black Mission and similar varieties are devoid of embryos, as are the induced parthenocarpic fruits of the Calimyrna. This source is probably the developing ovarian tissue, as pointed out by VAN OVERBEEK (**6**) and shown by MUIR (**5**), or the receptacular tissue.

It also is probable that figs which are normally parthenocarpic have a higher hormone content in the early stages of development than the non-parthenocarpic varieties, as shown by Gustafson for other fruits (**4**). This higher level is sufficient to initiate development, other sources becoming available as growth progresses. In the non-parthenocarpic varieties pollination, or externally added hormone, supply the initiating stimulus, following which an adequate hormone level is maintained as in a normally developing fruit. Apparently 2,4,5-T is so effective, either due to its chemical structure or factors of penetration, etc., that it not only initiates the growth reaction, but greatly accelerates the subsequent processes.

Summary

1. Results obtained during the 1948 season of investigations on the chemical induction of parthenocarpy in the Calimyrna fig are presented. Studies were made of nine chemicals applied as aqueous or oil emulsion sprays. These chemicals consisted of four chlorinated phenoxy acids, naphthalene and naphthoxy-acetic acids, and indole-acetic, propionic, and butyric acids.

2. Of these, indole-butyric, naphthalene-acetic, 2,4,5-trichlorophenoxyacetic, and para-chlorophenoxyacetic acid were effective in inducing parthenocarpic fruit development to maturity.

3. Indole-butyric acid, previously reported as active at 1500 p.p.m. was found to induce parthenocarpy at 200 p.p.m. Leaf chlorosis was evident at 600 p.p.m. or more. Naphthalene-acetic acid was increasingly effective from 25 to 250 p.p.m. It was slightly toxic at 50 p.p.m. and caused severe chlorosis at 250 p.p.m.

4. 2,4,5-trichlorophenoxyacetic acid induced parthenocarpy at a concentration as low as 10 p.p.m., and in addition greatly accelerated fruit maturation. Syconia sprayed with this material were judged mature on the

basis of size, color, texture, and flavor two weeks after treatment, in contrast to caprified fruit which required 75 days to reach a comparable stage of maturity. This compound also accelerated the maturation of caprified fruit and the normally parthenocarpic fruit of the Black Mission variety. It was also toxic, causing chlorosis at low concentrations and death in about a month at 75 and 100 p.p.m.

5. Para-chlorophenoxyacetic acid at 40, 60, and 80 p.p.m. resulted in a set of parthenocarpic fruit which matured normally and was essentially equal to the caprified controls in all respects. No injury was apparent from this material at these concentrations.

6. All parthenocarpic fruit was devoid of achenes and there was no sclerification of ovary walls. The flavor and color of the fresh fruit were equal to the caprified controls. The parthenocarpic fruit was slightly flattened dorsi-ventrally.

7. All parthenocarpic fruit was combined into a common lot, air dried, and processed and packed in the accepted commercial method. The resulting unit package was of good appearance and equal in weight to caprified fruit. A limited number of taste tests of the processed fruit indicated that the flavor was acceptable in most instances, although milder and somewhat different from similar caprified fruit.

8. Because of its relatively low cost, low required dosage, and lack of injurious effects, para-chlorophenoxyacetic acid appears to offer considerable promise as a means of eliminating the problems of caprification in the Calimyrna fig industry.

9. A brief discussion is included of the relationships of hormones to the growth of the fig fruit and to fruit growth in general.

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CHANGES IN THE CARBOHYDRATE METABOLISM OF *TARAXACUM KOK-SAGHYZ* ROD DURING THE FIRST AND SECOND YEARS OF GROWTH

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(WITH EIGHT FIGURES)

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An interest in kok-saghyz springs mainly from the presence of rubber in its roots, and there is evidence to believe that the synthesis of its rubber is intimately connected with carbohydrate metabolism. Thus, the greatest accumulation of both rubber and carbohydrates has been reported to occur simultaneously toward the end of the growing season (**8**). An inverse relation between these two substances has been reported during the period of summer rest (**7**). A direct synthesis of rubber from carbohydrates was observed in tau-saghyz seedlings grown on various sugars *in vitro* (**9**). It is reasonable, therefore, to expect that knowledge of carbohydrate metabolism of kok-saghyz would be valuable for the elucidation of the conditions affecting the synthesis of rubber.

One has also to bear in mind that kok-saghyz roots are very rich in carbohydrates. At its peak, carbohydrate content may be up to 18% of the root fresh weight. This compares favorably with up to 20% found in sugar beet, a plant which is cultivated specifically for the sake of its sugar. Kok-saghyz roots, therefore, represent a rich source of carbohydrates which might be useful to several industries. Their use in the alcohol industry has already been suggested (**1**), and since their main reserve has been reported to be inulin, they may also serve as a source of fructose. Again for successful industrial utilization of kok-saghyz roots, knowledge of their carbohydrate metabolism is highly desirable.

In spite of the fact that during the last 15 years kok-saghyz has been extensively studied, its carbohydrate metabolism so far has received only scant attention, and the occasional references to it are widely scattered throughout the literature. The work reported in this paper represents an attempt to remedy this situation, and to give a systematic picture of carbohydrate metabolism in this plant.

Materials and methods

On April 21, 1943, kok-saghyz seeds* were stratified and sown into flats. Seedlings were transferred from a greenhouse to a field on May 22. The first flowers appeared on June 30 and the peak of flowering was reached about the first week of August. This was followed by a marked diminution in the number of flowering plants, although occasional flowers were observed

*The author wishes to express his thanks to Dr. G. H. Duff, Department of Botany, University of Toronto, for his kindness in supplying these seeds.

even in September. No clear-cut summer rest was observed, although early in August a number of plants had some of their leaves turn temporarily brown and some had even withered away. In the fall, one half of the plants were dug, and the rest were left to winter in the field. In the case of the dug plants, leaves were cut off at their bases, and the roots were stored in sand during the following winter of 1943–44 in a cold storage room at 2–3° C. These stored roots, together with those dug periodically during the summer of 1943, provided the experimental material for plants in the first year of their growth. In this paper these plants will be referred to as "first-year plants, 1943–44." No analyses of their leaves were done.

Plants left in the field to winter survived to the extent of better than 95%. The first blossoms appeared on them early in May. Flowering reached its peak by June 1, and then declined. The earliest seeds were collected on May 24; the peak was reached on June 25, and it was followed by a rapid decline. After July 8, seed production became negligible. The first signs of summer rest were observed on June 21, and by August 9 close to 90% of all the plants were at rest. From the middle of August plants again began to produce leaves, and by October 1, the majority of them were in the vegetative stage.

Samples of these plants were taken throughout the growing season of 1944, and their roots and leaves were analyzed separately as described below. In the fall all of the remaining plants in the field were dug, their leaves were cut off and their roots were stored at 2–3° C as described above. During the winter of 1944–45, samples of these roots were periodically taken for analysis. Plants taken from this field in the summer of 1944, and from the storage in the winter of 1944–45 will be referred to as "second-year plants, 1944–45."

On May 18, 1944, another lot of seeds was stratified and sown directly into the field. The seeds used were those collected from the first-year plants, 1943–44. The first flowers appeared on July 12, and the peak of flowering was reached by August 20. No summer rest was observed. In the fall plants were dug and their roots were stored as described above. These plants will be referred to as "first-year plants, 1944–45." Both the leaves and roots of these plants were analyzed during the summer but only the roots during the winter.

The field on which plants were grown in both years was a fairly heavy clay. During the growing seasons inorganic fertilizers were applied along the rows every three to four weeks. These consisted of a mixture of superphosphate, potassium chloride and sodium nitrate. Rows were hoed and weeded as required. No serious infestations by any parasites were observed.

The technique of taking samples and preparing them for the analysis was as follows: A sample of usually 10–15 plants was brought to the laboratory, washed under the tap, and separated into roots and leaves. Correspondingly larger samples were taken of younger plants. The roots and leaves were wrapped separately with cheese cloth and frozen at −15° C in the ice

compartment of a refrigerator. While in the frozen state they were passed through a food chopper and ground to a fine pulp. This pulp was thoroughly mixed and a sample of it was taken for analysis.

Sugars were extracted from this pulp with 85% ethanol under a reflux condenser for 24 hours. The details of their extraction and the methods of their subsequent analyses are given elsewhere (**5**). Total sugars, reducing sugars and invert sugars were the three fractions determined. The invert sugars fraction was represented by the difference between the total sugars after inversion with HCl and the initial reducing sugar content. The results of all the analyses are expressed as glucose.

The alcohol-insoluble pulp residue was suspended in distilled water and kept in a boiling water bath for three hours under a reflux condenser. At the end of this time, it was filtered off and the filtrate was made to a volume. The reducing power of the filtrate was determined after inversion with HCl (**5**) and the results were expressed as glucose. In the present paper this fraction is called "inulin," and its chemical nature will be discussed later. In 1944–45 parallel analyses for the "inulin" fraction of the roots in the second year of growth were also done colorimetrically. The method used was based on the hydrolysis of "inulin" with HCl, and subsequent formation of the blue color between the fructose liberated and the diphenylamine added (**2**).

After the extraction of "inulin" from the alcohol-insoluble pulp, the residue was further extracted with 1% H_2SO_4 under a reflux condenser in a water bath. The extract obtained was filtered off, freed from H_2SO_4 with $Ba(OH)_2$, and analyzed for its reducing power. In the present paper this fraction is designated as hemicellulose. Both "inulin" and hemicellulose fractions were analyzed for their reducing power without being cleared with lead. Previous tests have indicated that such a clearing had no effect on the reducing power of these two fractions. In 1943–44 no separation was made between the "inulin" and hemicellulose. Instead, the alcohol-insoluble residue was extracted directly with 1% H_2SO_4, so that both "inulin" and hemicellulose fractions were combined. For the purpose of this paper they are designated as the "alcohol-insoluble fraction."

Results and discussion

Figures 1–3 give the changes in various carbohydrate fractions of kok-saghyz roots for the first-year plants, 1943–44, and figures 4–8 for the second-year plants, 1944–45. Data for the first-year plants, 1944–45 are omitted, since data for their roots are very similar to those for the first-year plants, 1943–44, and data for their leaves are essentially the same as for the second-year plants, 1944–45.

In the interpretation of these figures one should bear in mind the following salient characteristics of kok-saghyz ontogeny. In the spring growth is entirely vegetative, and the bulk of the dry matter produced by a plant goes into the production of leaves. In the first year of growth for the first

two months of the season, leaves constitute approximately 80% of the total plant weight (**8**). Appearance of flowers initiates a progressively increasing diversion of dry matter to roots, until by the end of the growing season in the first year of growth the absolute weights of leaves and roots are about equal. Appearance of flowers in increasing numbers is followed by the formation of fruits. In the first year of growth only a few fruits are formed, and their production is extended until early fall. There is no period of summer rest. In the second year of growth, fruit production is very heavy, and of relatively short duration. At the beginning of August, plants lose their leaves and enter a period of summer rest. A short resumption of vegetative activity in the fall is followed by a period of winter rest.

Figures 1 and 4 give data on the changes in the total carbohydrate content of the roots and on the relative importance of their respective alcohol-soluble and -insoluble fractions. An examination of these figures reveals that at the beginning of the growing season for both first and second years of growth, the percentage of total carbohydrates is low. It is, however, rapidly increasing, and for the roots in the first year of growth the highest value for the year is reached by the middle of the flowering period. In the second year of growth, after temporarily stabilized values during the period of intensive flowering and fruit formation, the upward trend is resumed, and the peak is reached shortly before the period of summer rest. In both years a decline from the peak is continued for the rest of the growing season and the period of winter rest. It is interrupted only by a brief secondary peak toward the end of the growing season. The initial drop from the peak value is particularly steep in the second year of growth, and it is temporarily stabilized during the period of summer rest.

The relative importance of the alcohol-soluble and -insoluble fractions in roots is not constant throughout the year, but undergoes a series of regular changes. The trends observed are essentially similar in both first and second years of growth. From the very beginning of the growing season, and until the resumption of vegetative growth in the fall, there is a progressive increase in the relative importance of the alcohol insoluble and a decrease in the alcohol soluble fraction of roots. In the second year of growth the relation reached toward the end of the fruiting period is temporarily stabilized during the period of summer rest. Resumption of growth by the middle of September reverses the trend, and toward the end of November close to 80% of the total carbohydrates are represented by the alcohol-soluble fraction. This relation is maintained throughout the whole period of winter rest.

Figures 2 and 5 give changes in the alcohol soluble fraction of roots. The drifts observed are essentially similar in both first and second years of growth. The main sugars to vary are invert sugars. They increase during the early vegetative growth reaching a peak by the middle of the flowering period. This is followed by a decline until the fall, when with the resumption of vegetative growth there occurs another rise, reaching a peak early in the period of winter rest. Subsequent decline continues throughout the

rest of the winter. Reducing sugars in roots are relatively constant. They are low throughout the main part of the growing season, rise shortly before the period of winter rest, and remain at this higher level throughout the winter.

From figure 6, as well as from the similar data obtained for the first-year plants 1944–45, it is evident that the hemicellulose content of roots remains practically constant throughout the whole ontogeny. It has about the same low value in both first and second years of growth. Any changes observed in the total alcohol insoluble fraction are those of "inulin." For this reason figure 3, which presents changes for the total alcohol insoluble fraction in the first-year roots, can be compared with the graph for "inulin" in the second-year roots as given in figure 6.

An examination of figures 3 and 6 reveals that in both first and second years of growth there is a steady increase in the "inulin" content of roots from the beginning of vegetative growth in the spring until the middle of the flowering period. For the first year of growth the "inulin" content reached at this time represents the peak value for the year, and this is maintained relatively constant until shortly before the onset of the winter rest. Then it rapidly declines, coming to low, steady values at the beginning of the winter and these are maintained throughout the rest of the winter. In the second year of growth, accumulation of "inulin" is only temporarily suspended during the peak of flowering and fruit formation, probably because of a heavy demand on sugars during this period. As soon as fruit formation is over, accumulation of "inulin" is resumed once more and the peak value for the year is reached shortly before the onset of the summer rest. This is followed by a precipitous drop which is temporarily stabilized, still at high values, during the period of summer rest. With the resumption of growth in the fall, "inulin" content again begins to go down, but the steady, low winter values are reached only after three to four months of decline.

Figure 6 also shows the results of "inulin" analyses in which the colorimetric method was used. An examination of this figure reveals that early and late in plant ontogeny both methods of analysis yield comparable results. This is not so, however, for the middle of the growing season, immediately preceding and during the period of summer rest. During this period colorimetric method yields considerably higher results. This discrepancy between both methods suggests that the so-called "inulin" fraction in koksaghyz is not a homogeneous one throughout the whole plant ontogeny.

Figures 7 and 8 give changes in the alcohol soluble and insoluble fractions of leaves in the second year of growth. Essentially the same results were obtained for plants in the first year of growth. The main carbohydrate fraction in leaves is represented by sugars. They increase during the early part of the growing season, reaching a peak about the middle of the flowering period. From this time on they decline. The alcohol-insoluble fraction is low and relatively constant throughout the whole season.

Several attempts have been made to isolate inulin from the first-year roots dug in September. Neither the freezing method of SPOEHR (**10**), nor crystallization in cold from 75% ethyl alcohol produced anything but minute quantities of inulin. The bulk of the "inulin" fraction remained in the solution. It is clear, therefore, that in kok-saghyz the so-called "inulin" fraction is not represented by inulin alone, but consists of at least two different components. Both are insoluble in 85% ethanol and soluble in hot water. The first component is true inulin, as can be inferred from its general appearance after precipitation in cold from 75% ethanol and from its melting point determination. It forms, however, only a small portion of the "inulin" fraction. The second component, which forms the bulk of this fraction, is closer to the "89% ethanol insoluble levulins" as described by TRAUB and SLATTERY for guayule (**11**). A predominance of levulins over "inulin" is, therefore, a characteristic common to both kok-saghyz and guayule.

That the "inulin" and alcohol soluble fractions are mutually interconvertible has been reported for common dandelion (**6**), and one might expect, therefore, to find the same in kok-saghyz. An examination of figures 2 and 5, reveals that there is a rise in the sugar content of roots toward the end of the growing season. This rise sets in with the resumption of vegetative growth in the fall before roots were dug, and it continues at about the same rate after placing the roots in storage. For this reason, one can assume that this rise is a normal phenomenon, characteristic of later stages of growth and is not an artifact produced by digging. Since in the initial stages of this rise, at least in the first year of growth, the "inulin" content remains fairly constant, some other fractions besides "inulin" must be producing sugars. It is interesting to note that this rise is caused mainly by the accumulation of invert and not of reducing sugars.

That under some conditions large amounts of sugars may unexpectedly appear in plant tissues has already been reported by several workers. This has been observed in mature mangold (**3**) and wheat (**4**) leaves at night, when appearance of sugars as a result of photosynthesis is excluded. This has also been found in apple fruits toward the end of the growing season (**5**). The total amounts of sugars appearing under these conditions is considerable. In the case of mangold leaves the sugar content at night may be higher than during the peak of photosynthesis. In the case of McIntosh apples approximately one quarter of their sugars present at maturity come from this source, which at the present time is unknown. In all these cases, as in kok-saghyz, the rise is caused mainly by invert and not by reducing sugars.

As is seen from figures 3 and 6 the greatest decrease in the "inulin" fraction of roots occurs immediately preceding winter and summer periods of rest. On the assumption that "inulin" and sugar fractions are mutually interchangeable, one expects that a decline of "inulin" must be accompanied by a corresponding rise in sugars. A comparison between figures 2 and 3,

5 and 6 reveals that while in the fall disappearance of "inulin" is accompanied by an increase in sugars, the magnitude of their rise is below that which might be expected. Moreover, as seen from figures 5 and 6, a decline from the peak values in "inulin" previous to the summer rest is accompanied not by an increase but by a decrease in the sugar content. Table I gives a balance sheet for the amounts of "inulin" which disappeared and sugars which accumulated as observed in various experiments.

An examination of table I reveals that the disappearance of the "inulin" fraction from the roots may or may not be accompanied by a corresponding accumulation of sugars. The disappearance of "inulin" immediately preceding summer rest is accompanied not by an accumulation but instead by a

TABLE I

DISAPPEARANCE OF "INULIN" FRACTION AND ACCUMULATION OF SUGARS IN THE PERIODS IMMEDIATELY PRECEDING WINTER AND SUMMER REST. (AS GMS. OF GLUCOSE PER 100 GMS. ROOT FRESH WEIGHT)

	"INULIN" FRACTION BY REDUCING POWER	"INULIN" FRACTION COLORIMETRICALLY	TOTAL SUGARS
1943–44 first year			
July 10–Aug. 16	−1.8	.....	−3.5
Sept. 29–Nov. 15	−6.8	.....	+3.2
1944–45 first year			
Oct. 21–Nov. 15	−7.8	.....	+4.7
1944–45 second year			
Aug. 7–Aug. 15	−2.6	−6.6	−0.7
Sept. 12–Nov. 15	−4.7	−5.4	+4.1

decline in the sugar content. Preceding winter rest, even if there is a simultaneous accumulation of sugars, the amounts formed are only a fraction of those which might be expected. All this evidence points to the conclusion that preceding summer rest and at the beginning of the winter, large amounts of the "inulin" fraction are transformed into some form other than sugars. Previous to the period of summer rest sugars disappear from the roots and are transformed into some form other than "inulin."

Rapid, unexplained disappearance of sugars from plant tissues in amounts far in excess of those used in respiration have already been reported. This behavior was observed under the conditions of brief starvation in mature wheat leaves taken at definite periods of day or night (**4**). It was also evident in apple fruits at the beginning of the winter storage (**5**). In both these cases the organs used were fully developed, so the possibility of new cell formation was excluded.

Since this unaccountable appearance and disappearance of carbohydrates has been observed in such diversified organs as leaves, fruits and roots, one has to assume that these represent normal stages of plant metabolism, which so far have been overlooked. Since the amounts involved in such transformations are large, constituting up to one half of the total carbohydrates present, they cannot be neglected. It is clear, therefore, that investigations

of the carbohydrate metabolism of a plant, based on carbohydrate analyses alone, cannot give us a complete picture. Such studies must also take into account the metabolism of substance or substances which in definite stages of plant ontogeny either break down producing large quantities of sugars, or are synthesized and in this way serve as a sink into which sugars or other forms of carbohydrates disappear.

Summary

1. The bulk of carbohydrates in kok-saghyz are found in its roots, where their concentration at the peak of the season is six to eight times that of leaves.

2. During the growing season alcohol-soluble carbohydrates predominate in leaves, while alcohol-insoluble ones are characteristic of roots.

3. The alcohol insoluble carbohydrates of roots consist mainly of levulins similar to those described for guayule. Inulin is present only in small amounts.

4. In roots large amounts of sugars appear toward the end of the growing season, and large amounts of the alcohol insoluble carbohydrates disappear at the beginning of the summer and winter rest periods. This appearance of sugars or disappearance of the alcohol-insoluble carbohydrates is not due to the mutual transformations of these two fractions. They involve some other substance or substances, whose chemical nature is unknown at present.

5. Since similar large scale disappearance and appearance of sugars has been reported for a number of different plants and plant organs, it is concluded that these constitute normal, though not yet recognized, phases of carbohydrate metabolism in plants.

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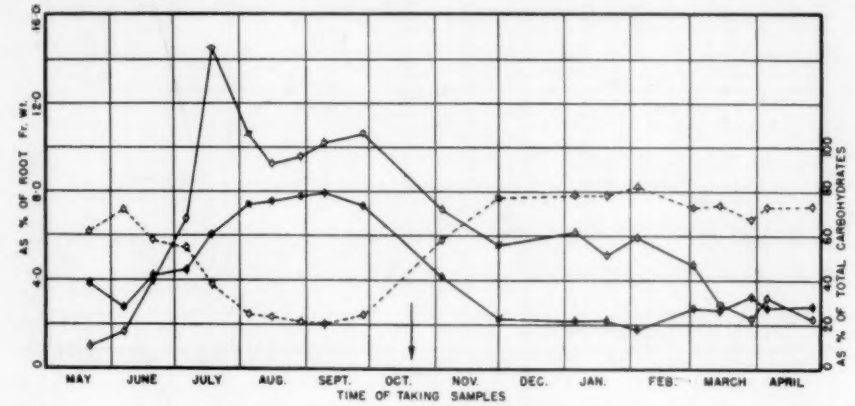

FIG. 1. Seasonal changes in the total carbohydrate content of roots in the first year of growth, 1943–44.

Total carbohydrates —○——○—; alcohol soluble fraction —○- - -○—;

Plants dug ↓ ; alcohol insoluble fraction —●——●—;

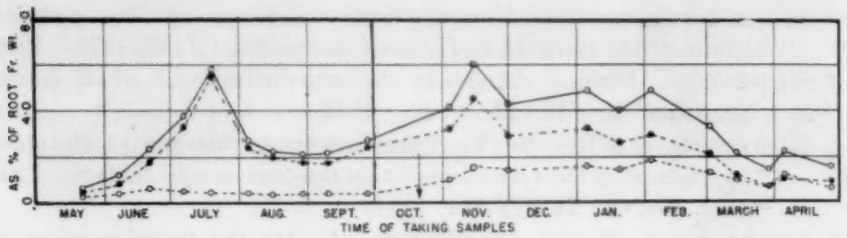

FIG. 2. Seasonal changes in the alcohol soluble fraction of roots in the first year of growth, 1943–44.

Total sugars –○———○–; invert sugars –●- - - -●–;
Reducing sugars –○- - - -○–; plants dug ↓ ;

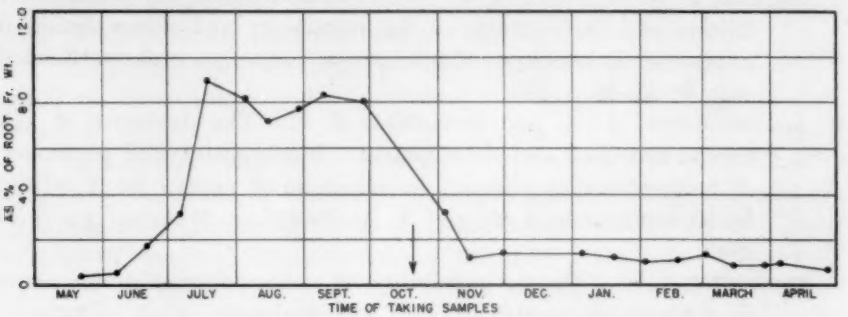

FIG. 3. Seasonal changes in the alcohol insoluble fraction of roots in the first year of growth, 1943–44.

The sum of the "inulin" and hemicellulose fractions –●———●–;
Plants dug ↓ ;

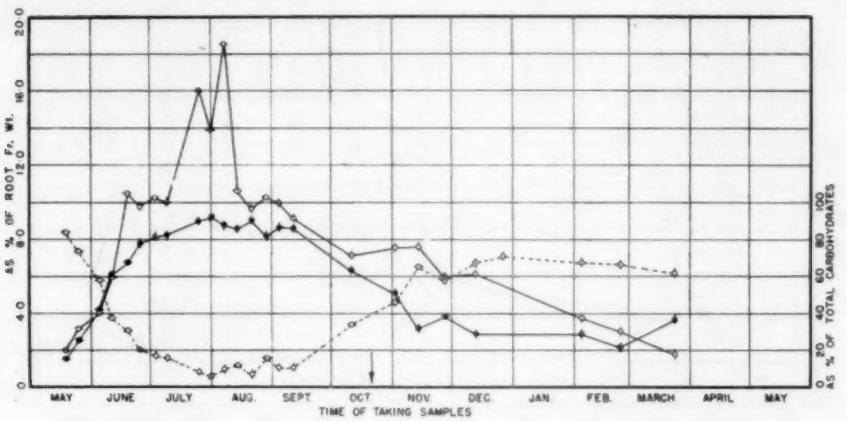

FIG. 4. Seasonal changes in the total carbohydrate content of roots in the second year of growth, 1944–45.

Total carbohydrates –○———○–; alcohol soluble fraction –○- - -○–;
Plants dug ↓ ; alcohol insoluble fractions –●———●–;

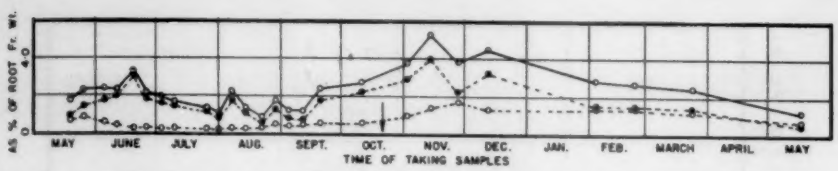

FIG. 5. Seasonal changes in the alcohol soluble fraction of roots in the second year of growth, 1944–45.

Total sugars -○———○-; invert sugars -●- - - -●-;
Reducing sugars -○- - - -○-; plants dug ↓ ;

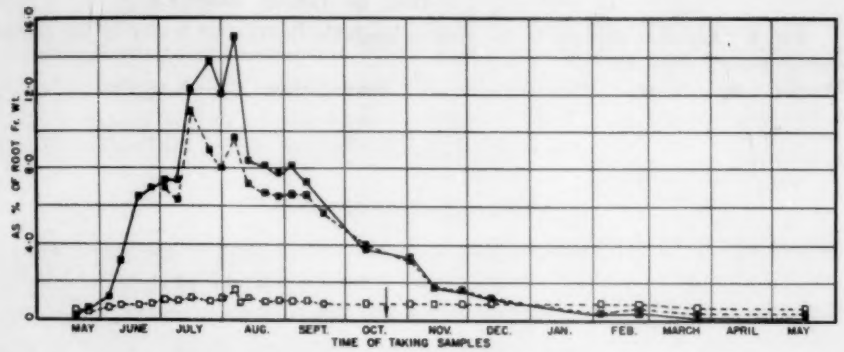

FIG. 6. Seasonal changes in the alcohol insoluble fraction of roots in the second year of growth, 1944–45.

"Inulin" from reducing power -■- - - -■-; inulin from colorimetric determination -■———■-;
Plants dug ↓ ; hemicelluloses -□- - - -□-;

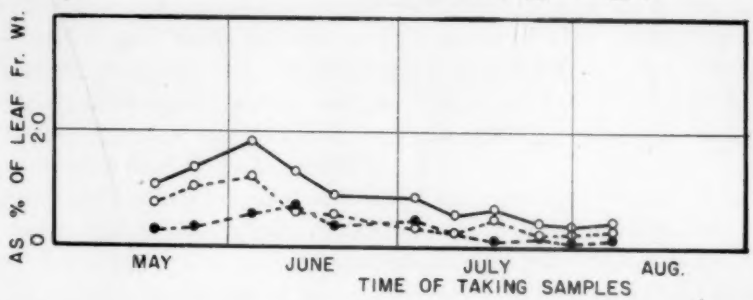

FIG. 7. Seasonal changes in the alcohol soluble fraction of leaves in the second year of growth, 1944–45.

Total sugars -○———○-; invert sugars -●- - - -●-;
Reducing sugars -○- - - -○ ;

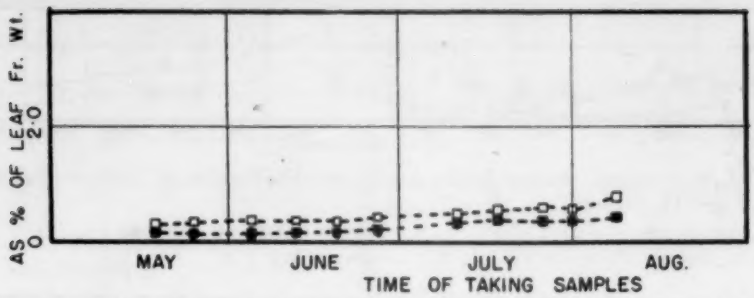

FIG. 8. Seasonal changes in the alcohol insoluble fraction of leaves in the second year of growth, 1944–45.

"Inulin" -■- - - -■-; hemicelluloses -□- - - -□-;

ARSENATE AS A SELECTIVE INHIBITOR OF GROWTH SUBSTANCE ACTION

JAMES BONNER

Received August 6, 1949

The action of growth substances in causing cell elongation is closely linked to respiration, and many respiratory inhibitors also inhibit growth (COMMONER and THIMANN, **3**, BONNER, **1, 2**). In the study of the way in which growth substances act to bring about growth it is therefore useful to have inhibitors which will suppress the action of growth substances without affecting respiration of the treated tissue. Such a selective inhibitor of growth substance action has been found in arsenate.

Materials and methods

The techniques used in this investigation were similar to those described in earlier papers (**1, 2**). Avena seeds of the variety Sieges-hafer were planted in vermiculite and allowed to germinate at 25° C under occasional orange light. When the coleoptiles had attained a height of approximately 2 cm. they were cut from the roots, decapitated and sections 5.0 mm. long cut from the apical end of each coleoptile with a double bladed cutting tool. For growth measurements sections were incubated at 26° in darkness for 24 hours in 20 cc. of the desired solution contained in Petri dishes. The solutions contained in all cases 2% sucrose and 0.0025 m. maleate buffer, pH 4.5. Indole-acetic acid was added in concentrations of 3 or 10 mg/liter. All addenda of phosphate and arsenate were adjusted to pH 4.5 before use. Twenty sections were used in each portion of each experiment and each experiment repeated two to 10 times. The results reported in this paper are based on a total of 24 experiments.

Respiration measurements were made manometrically using 16 cc. conical vessels with central alkali wells. For these measurements, 20 sections were suspended in 2 cc. of the desired solution and measurements of gas exchange made over a period of several hours. The data recorded in this paper are all taken from the third hour after initiation of the experiment.

Experimental results

The growth of excised sections of Avena coleoptiles in solutions of indole-acetic acid (IAA) is inhibited more than 50% by sodium arsenate in a concentration of approximately 10^{-5} molal and is 90% inhibited by approximately three times this concentration. The rate of endogenous (no added substrate) respiration of Avena coleoptiles is unaffected by arsenate up to a concentration of 10^{-3} molal over a period of several hours, as is shown in table I.

TABLE I

EFFECT OF SODIUM ARSENATE ON GROWTH AND RESPIRATION OF AVENA COLEOPTILE SECTIONS. ALL SOLUTIONS 2% IN SUCROSE AND BUFFERED AT pH 4.5 WITH .0025 MALEATE BUFFER

CONCENTRATION OF SODIUM ARSENATE MG/L	RATE OF O_2 UPTAKE MM^3 O_2/100 MG. FRESH WT/HR.	GROWTH IN IAA, 3 MG/L IN 24 HOURS % OF IAA CONTROL	% INHIBITION OF GROWTH
0	35	100	.....
1	.....	60	40
3	.....	36	64
10	36	12	88
30	35	6	94
100	35	.....	.....

It is known from investigations on isolated enzyme systems (NEEDHAM and PILLAI, **5**, WARBURG and CHRISTIAN, **6**) that arsenate is able to substitute for inorganic phosphate in oxidative reactions for which inorganic phosphate uptake is otherwise an essential feature of the reaction mechanism. Thus in the presence of arsenate, substrate oxidation is enabled to proceed without the production of energy-rich phosphate. That arsenate inhibition of growth substance action is likewise associated with phosphate metabolism is indicated by the data of table II which show that arsenate

TABLE II

REVERSAL OF ARSENATE INHIBITION BY ADDED PHOSPHATE. AVENA SECTIONS GROWN IN 2% SUCROSE, 0.0025 IN MALEATE BUFFER, FOR 24 HOURS. IAA = 3 MG/L.

ARSENATE CONC. MG/L	ADDED PHOSPHATE, MOLAL			
	NONE	5×10^{-4}	5×10^{-3}	5×10^{-2}
	GROWTH IN % OF IAA CONTROLS (NO ARSENATE)			
0	100	100	100	100
1	60	96	96	103
3	36	82	92	106
10	12	26	52	99
30	6	11	10	71

inhibition is overcome by addition of phosphate. The effect of phosphate in overcoming the inhibitory influence of arsenate does not appear to be simply due to effects of one ion upon the uptake of the other into the tissue. Thus the presence of arsenate did not depress the uptake of radioactive inorganic phosphate even when phosphate was present in smaller concentration than the arsenate.

Indole-acetic acid increases the rate of respiration of Avena coleoptile sections by some 25–35% (**2, 3**). The increased respiration like the growth-promoting effect of IAA is inhibited by arsenate as is shown in table III. In the presence of arsenate the IAA-induced respiratory increase is abolished although the endogenous respiration is unaffected. It has previously been

TABLE III

ARSENATE INHIBITION OF IAA-INDUCED INCREASE IN RESPIRATION. EXCISED AVENA COLEOPTILE SECTIONS IN 2% SUCROSE, MALEATE BUFFER, pH 4.5

IAA MG/L	ARSENATE MG/L	MM^3 O_2/100 MG. FRESH WT/HR.	% INCREASE ABOVE CONTROL
0	0	33	.....
10	0	44	33
0	100	35	6
10	100	33	0

shown (BONNER, **2**) that adenylic acid and arginine like IAA increase respiratory rate of Avena coleoptile sections. These increases likewise are inhibited by arsenate.

Discussion

Arsenate, well known as an inhibitor of phosphate metabolism, is an inhibitor of the growth and respiratory effects of IAA on excised sections of Avena coleoptiles. That the effect of arsenate on such sections is through an effect on phosphate metabolism is indicated by the fact that arsenate inhibition is offset by added phosphate. These results suggest then that IAA affects growth and respiration through a system which involves the metabolism of phosphate. A similar interpretation has been made earlier (**1, 2**) of experiments involving 2,4-dinitrophenol as an inhibitor for the growth substance reaction. Dinitrophenol, like arsenate, is known from enzymatic work to bring about the uncoupling of oxidative reactions from the associated energy-rich phosphate storing mechanism. Dinitrophenol like arsenate inhibits growth substance action in the Avena coleoptile without any detectable inhibition of endogenous respiration.

Summary

1. The indole-acetic acid-induced growth of excised Avena coleoptile sections is inhibited by arsenate in the range of 10^{-5}–10^{-4} molal. These concentrations of arsenate do not inhibit the endogenous respiration of the same tissue but do inhibit the indole-acetic acid-induced increase in respiration.

2. The inhibitory effect of arsenate on indole-acetic acid-induced growth is reversed by added inorganic phosphate. This suggests that arsenate may exert its inhibitory effect on growth through an influence on phosphate metabolism.

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SIMPLIFIED EQUIPMENT FOR SUBIRRIGATION EXPERIMENTS IN PLANT NUTRITION

GEORGE R. JOHNSTONE

(WITH ONE FIGURE)

Received September 29, 1949

The two well known techniques which are used in plant-nutrition studies are conveniently designated the solution-culture method and the sand-culture method. Some other slightly soluble materials, such as cinders, gravel, vermiculite, and Sponge Rok or various combinations of these may be substituted for sand. The use of slightly soluble material as a medium, to which the nutrient solution is properly added, has certain advantages over the strictly solution-culture method. Good aeration and anchorage are provided in the sand-culture technique.

It is the present purpose to describe simplified equipment for subirrigation experiments in plant nutrition. This equipment can be assembled from miscellaneous materials at a minimum cost. Tin containers (fig. 1) properly selected as to size, shape and volume, so that one (A) can be superimposed above the other (F), thus making a two-compartment container, are desira-

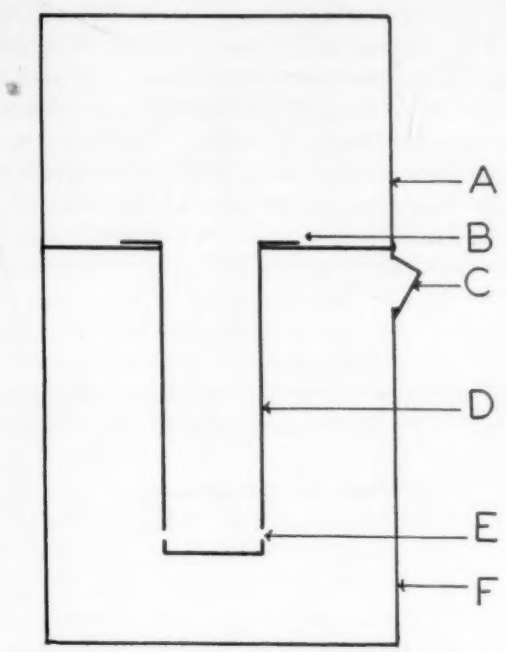

FIG. 1. A double-compartment container for subirrigation experiments in plant nutrition. A, upper compartment; B, plastic collar; C, opening for use in filling the lower compartment; D, plastic tube; E, perforation; F, lower compartment.

ble. A centered, circular hole about 1¼ inches in diameter is cut in the bottom of the upper compartment (A). In this opening, a plastic tube (D) of about the same diameter is placed. The upper end of this plastic tube is fitted with a plastic collar (B) to hold the tube in place with its lower end almost touching the bottom of the lower compartment (F). The lower end of the plastic tube needs four or five openings or perforations (E) about ⅛ inch in diameter. These perforations can be made with a strong paper punch or by inserting a redhot needle through the wall of the tube. The lower end of the tube is then sealed with a plastic cap of material such as cellulose acetate. An opening (C) with or without a lip is convenient for adding water. The two tin compartments are coated inside with inert asphalt paint to prevent reaction of the chemicals of the nutrient solution with the tin or other metallic substance which may be present in the container wall. After the plastic tube has been prepared and placed in position in the bottom of the upper compartment, the upper compartment is filled together with the plastic tube with slightly soluble material, such as pure-quartz sand, Sponge Rok, or vermiculite, etc., depending upon the degree of purity desired. The lower compartment is filled with the nutrient solution to a desirable level allowing for the volume of the plastic tube and its contents of slightly soluble material. The function of the plastic tube with its contents is to conduct the nutrient solution by capillarity and diffusion to the slightly soluble material in the upper compartment. This system is ideal in many respects, resembling or approximating conditions which often occur in nature. The combination of plastic tubing, river sand and glazed-clay, double compartment containers have been used successfully for 18 months in the culture of African violets, *Saintpaulia ionantha*, and for the period of one semester with *Impatiens sultani*. Tap water or distilled water must be added to the lower compartment (C) once or twice a week to replace that which is lost by transpiration and/or evaporation.

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NOTES

Southern Section Meeting.—The Southern Section of the American Society of Plant Physiologists will hold a joint meeting with the American Society for Horticultural Science at Biloxi, Mississippi, in connection with the annual convention of the Association of Southern Agricultural Workers, February 9, 10, and 11, 1950. The joint session will be devoted to discussion of Systems of Mineral Deficiency Diagnosis, under the chairmanship of Dr. H. C. Harris. The half-day joint program comprises the following papers: By Soil Tests, I. E. Miles (Agronomy) and C. G. Milne (Horticulture); By Tissue Tests, G. N. Hoffer (Agronomy) and E. M. Emmert (Horticulture); By Visual Deficiency Symptoms, Leland Scott; Coordination of all Three Systems of Diagnosis, George Scarseth.

J. E. Weaver, Honorary President, Seventh International Botanical Congress.—Stockholm, 1950.

The American Society of Plant Physiologists is proud to announce that another of its members, Dr. J. E. Weaver, has been appointed an Honorary President of the Seventh International Botanical Congress to be held in Stockholm, Sweden, next year. Dr. Weaver has been a member of the American Society of Plant Physiologists since its beginning. He was Research Associate at Carnegie Institution from 1922 to 1930 and has been Professor of Plant Ecology at the University of Nebraska since 1917.

Frank J. Thone.—Dr. Thone, 58, biology editor of Science Service and well known science reporter for the past quarter century, died August 25 of coronary occlusion in Washington, D. C. He was born in Davenport, Iowa, in 1891. He attended Grinnell College until 1915, then the University of California, Johns Hopkins University, and received the Ph.D. from the University of Chicago in 1922. Dr. Thone taught at North Dakota State College and the University of Florida before joining the staff of Science Service in 1924. A Charter Member of the American Society of Plant Physiologists, he was a specialist in plant ecology and in 1946 received a Westinghouse award for distinguished science writing. He is the author of TREES AND FLOWERS OF YELLOWSTONE NATIONAL PARK and THE MICROSCOPIC WORLD.

Principles of Field Crop Production.—J. H. MARTIN and W. H. LEONARD. The Macmillan Company, 60 Fifth Avenue, New York. 1176 pages, 1949. $6.00.

This book is designed as a college text for a general course in field crops, especially for agricultural students who take only one course in crop production. The book presents some of the facts and fundamental principles essential to an understanding of field crop production in the United States.

It contains more material than can usually be covered in a one-semester course, but it offers a choice of subject matter to meet different institutional and local requirements. Some knowledge of botany and chemistry is desirable but not essential to an understanding of the material presented. The seven main parts of the book include: General principles of crop production; Crops of the grass family; Legumes; Crops of other plant families; Appendix; Glossary; and Index.

Physical Methods of Organic Chemistry—Part I.—ARNOLD WEISSBERGER, Editor. Second Edition. Interscience Publishers, 215 Fourth Avenue, New York 3, New York. 1072 pages, 1949. $12.50.

The second edition of this well known volume has been enlarged by five new chapters and by a substantial expansion of many of the other chapters; some of them have been entirely rewritten and others have been thoroughly revised. This series is devoted to a comprehensive presentation of the techniques which are used in the organic laboratory and which are available for the investigation of organic compounds. The authors give the theoretical background for an understanding of the various methods and operations and describe the techniques and tools, their modifications, their merits and limitations, and their handling. The present work has been compiled with the hope of relieving the scientist of searching through periodicals and specialized books in order to acquaint himself with a certain physical method. It has been the object of the authors to provide a description of tested methods, the theoretical background for understanding and handling them, and the information necessary for a critical evaluation of the experimental results.

Le Bouturage et les Substances de Croissance Synthetiques. E. J. B. VERLEYEN. H. Wellens and W. Godenne, Brussels. 198 pages, 1948.

This publication is a dissertation for the Doctor of Science degree in the University of Louvain. It consists of a discussion of the morphological, physiological and environmental factors involved in the rooting of cuttings and a survey of the literature pertaining to the problem. The bibliography includes 484 references. Reports are given for extensive experiments on the rooting of cuttings of some eighty varieties of horticultural plants about half of which are ornamental Conifers. The results are presented in detail in 85 tables and 37 plates and include the effects of various concentrations of β indoleacetic acid and α naphthaleneacetic acid applied in aqueous solutions. The monograph is summarized in seven languages.

Molecules and Crystals in Inorganic Chemistry.—A. E. VAN ARKEL. Interscience Publishers, 215 Fourth Avenue, New York 3, New York. 234 pages, 1949. $3.85.

This book is devoted almost entirely to the subject of the correlation of structure and properties that can be derived from considerations of the

heteropolar bond. The author believes that a thorough grasp of all the implications of the heteropolar bond provides the student with a wide insight into inorganic chemistry as a whole. Two conceptions of molecular aggregation are dealt with. The parts of the book are as follows: Introduction; The chemical bond; The ionic bond; Properties of ionic compounds; Chemical reactions; Complex compounds; Polarization; Water as a component of compounds and solutions; Non-electrostatic bonds; Table of known elements; for further reading; Index; and Appendix.

PREPARATION OF MANUSCRIPTS FOR PUBLICATION IN PLANT PHYSIOLOGY

The following suggestions are made to authors writing papers for this journal in order to minimize revisions and editorial corrections. Though many points may appear arbitrary or trivial, attention to details is necessary to secure uniformity of format and style. The membership dues and subscription fees go almost entirely to publication costs, as all of our editors give their service without financial recompense. Hence the cooperation of authors in preparation of manuscripts minimizes expense and permits greater liberality as to length and number of papers published. Authors will find the following concise publications useful in preparation of manuscript copy: A. J. Riker, The Preparation of Manuscripts for Phytopathology. Phytopathology XXXVI, pages 953–977, 1946; and S. F. Trelease, The Scientific Paper: How to Prepare It. Williams and Wilkins Company, Baltimore, Maryland; 1947.

1. **Length.** The allowable length of papers is 20 printed pages, with tables and figures not to exceed 25 per cent of the total. Authors will be billed at cost for overrun and excess of figures and tables.

2. **Format.** In the preparation of typescripts, authors are requested to examine a printed copy of the journal of PLANT PHYSIOLOGY to note the position of headings, general organization, punctuation, abbreviations, and the methods of citing literature particularly, in order to bring the script into conformity with the general style of the journal. Leave a 3-inch margin at the top of the first page, liberal side margins, and double spacing of lines to permit proofmarking and required editorial changes.

3. **Headings.** Leave all headings unscored and scientific names without underlining, as the editors will proofmark these items in accordance with the practice of the journal. Generic names used alone are usually capitalized but not italicized. In general, do not underscore any lines.

4. **Footnotes.** Avoid footnotes as far as possible by placing such items under acknowledgments or at end of summary. When footnotes are used, they should be numbered with Arabic numerals.

5. **Figures.** Copy for figures (photographs and drawings) should not exceed $8\frac{1}{2} \times 11$ inches. Retain original drawings as a safeguard against loss. If the figures submitted are larger than $8\frac{1}{2} \times 11$, they will be photographed at this office and the author will be billed for the same. Author's name and figure number must be written on the back of copy for each figure. Do *not* put legends into or on the figure, either front or back. Type all legends *double* spaced, properly numbered to correspond to the figures, on a *separate sheet* of your manuscript. Graphs and drawings should be inked with fairly heavy lines and with lettering of approximately uniform size to insure clarity of printed figures after reduction. Photographic prints should be submitted on gloss-finish paper. Limit copy to desired visual details, trim off irrelevant foreground and minimize blank space, as charges for cuts are based on area. Unless otherwise directed, the editor will proofmark copy to determine actual size of printed figures. Old cuts will be sent to authors after printing for cost of postage if printer is so directed when galley proof is submitted.

6. **Tables.** Place each table on a separate page; do not crowd too much material into a single table. Note size of printed page (width and length, $4\frac{1}{2} \times 7\frac{1}{2}$

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7. **Acknowledgements.** Place acknowledgements at the end of the paper just below summary.

8. **Address.** The name of your institution and its address should also be given at the end of the summary.

9. **Literature Cited.** Use separate pages for the literature cited and type *double spaced*. Give complete citations, author, title, journal name, volume number, inclusive pages, and year. (See any literature list in a recent issue of the journal for examples.) Punctuate according to the examples, and leave no citations incomplete. Write out names of states in full. Note proper abbreviations of journals. An error frequently noted in manuscripts with long bibliographies is failure to mention in the text all citations listed. Authors are requested to check all citations in the bibliography by careful proofreading of manuscripts prior to submission.

10. **Formulae.** Use mathematical formulae only when necessary, and be conservative in the use of space.

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14. **Abstracts.** Authors are requested to submit typed abstract (blue form) when corrected galley is returned to editor who will insert proper volume and paging for use by Biological Abstracts. If authors care to type a carbon copy of abstract on plain paper, the second abstract will be sent to Chemical Abstracts or some other suitable abstract journal.

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Manuscripts for Plant Physiology should be addressed to Prof. Walter F. Loehwing, Department of Botany, State University of Iowa, Iowa City, Iowa. Drawings and photographs should not exceed 8×11.5 inches in size, should be numbered correctly on the back, and separate typewritten legends included with the text of the manuscript. Tables and cuts should be used sparingly, and the text presented in concise form. Instructions to authors for preparation of manuscripts are given in the January number, Volume 24, 1949, of Plant Physiology, pages 192–194.

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